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# CONTENTS

## JANUARY. No. 1

The Effect of the Ages of Sire and Dam on the Average Butterfat Production of Offspring in Dairy Cattle. Warren Gifford and Erwin C. Elting....	1
The Freezing Point of Cheddar Cheese: Injury of Cheese by Freezing. H. H. Sommer.....	9
A Study of the Yellow Cocci Which Survive Pasteurization. B. W. Hammer and G. Malcolm Trout.....	18
Studies in the Normal Depositions of Minerals in the Bones of Dairy Calves. J. H. Kruger and S. I. Bechdel.....	24
The First Courses in Dairy Husbandry. H. P. Davis.....	35
Bacterial Action in the Coagulation of Evaporated Milk. W. A. Cordes...	46
Variability in Composition of Butter from the Same Churning in Relation to Working. V. C. Manhart.....	52
Butter Versus Oleomargarine in Ricket Control in Pigs. Ernest L. Anthony..	66
A Comparison of Three Methods of Pasteurizing Milk for Cheddar Cheese-Making. Walter V. Price and Paul S. Prickett.....	69
The Possible Toxicity of Buttermilk Soured in Zinc Containers. A. D. Burke, Frank Woodson and V. G. Heller.....	79
Influence of Temperature on the Changes Produced in Milk by Certain Bacteria. R. S. Sarkaria and B. W. Hammer.....	89
Abstracts of Some Papers Presented at the Annual Meeting.....	102
Special Meeting of the American Dairy Science Association.....	111
Announcement.....	116
Dairy Notes.....	117

## MARCH. No. 2

Calf Meal Studies. I. Laboratory Experiments in the Improvement of Physical Condition. II. Feeding Experiments with Cooked and Uncooked Meal. J. G. Archibald.....	119
The Ice Cream Scoring Contest as a Means of Improving the Quality of Ice Cream. A. C. Fay and W. H. Martin.....	136
The Keeping Qualities of Ghee. Vishram H. Patil and B. W. Hammer....	143
A Method of Awarding Marks for Uniformity of Low Bacterial Count in Clean Milk Competitions. Cowper H. Chalmers.....	155
A Method of Obtaining Crude Milk Sugar and Other Solids from Sweet Whey. R. W. Bell, P. N. Peter, Wm. T. Johnson, Jr.....	163

## MAY. No. 3

Gassy Fermentations in Reheated or Processed Cheese Products Containing Pimentos. W. R. Albus and S. Henry Ayers.....	175
The Value of Silage in the Experimental Ration. H. T. Converse.....	179

Physical Factors Influencing the Formation and Fat Content of Gravity Cream. H. C. Troy and Paul Francis Sharp.....	189
The Activity of the Mammary Gland as Determined by Analysis of Mammary Blood Before and After Milking. E. A. Cockefair.....	230
The Determination of Moisture in Dry Skim Milk by the Bidwell-Sterling Toluene-Distillation Method. P. A. Wright.....	240
The Heat Stability and Feathering of Sweet Cream, as Affected by Different Homogenization Pressures and Different Temperatures of Forewarming. Byron H. Webb and George E. Holm.....	243

## JULY. No. 4

Physical Constants of the Milk as Influencing the Centrifugal Separation of Cream at Various Temperatures. Paul Francis Sharp.....	259
Growth in Weight of Guernsey Cows After the Age of Two Years. Charles W. Turner.....	265
Lipins and Sterols as Sources of Error in the Estimation of Fat in Buttermilk by Ether Extraction Methods. L. M. Thurston and W. E. Petersen...	270
A Comparison of Certain Methods for Determining the Sanitary Quality of Ice Cream. John Weinzirl and L. S. Harris.....	284
The Effect of Flash Pasteurization of Milk upon the Flavor and Texture of Cheddar Cheese. C. A. Phillips.....	292
Effect of Homogenizing Ice Cream Mixes Before and After the Addition of Gelatin or Sugar and Before and After Condensing. J. C. Henning.....	299
The Effect of Hydrogen Ion Concentration on the Bacterial Content of Gelatin. A. C. Fay.....	313
A Comparison of the Volatile-Solvent Method with the Vacuum-Oven Method for Determining Moisture Content of Cheese. George P. Sanders.....	325
Book Reviews.....	331

## SEPTEMBER. No. 5

Studies on Butter Salts. O. F. Hunsiker, W. A. Cordes, and B. H. Nissen.....	333
Destruction of Botulinum Toxin by Milk Bacteria. J. M. Sherman, C. N. Stark, and Pauline Stark.....	352
Bovine Infectious Abortion. Increased Productivity of an Abortion-Free Dairy Herd. George C. White, Robert E. Johnson, Leo F. Rettger and James G. McAlpine.....	359
A Defect in Milk Due to Light. William C. Frazier.....	375
Some Observations on the Consistency of Cream and Ice Cream Mixtures. George Monroe Bateman and Paul Francis Sharp.....	380
A Study of the "Common White" Yeasts Found in Dairy Products. J. A. Nelson.....	397
The Influence of the pH of Agar Media Upon the Bacterial Counts of Raw and Pasteurized Milk. Jack Faber.....	401
The Bacterial Content of Orange Sherbert. A. C. Fay.....	404
The Volume of the Cream Layers Forming on Holstein and Jersey Milk. J. C. Marquardt and A. C. Dahlberg.....	409
The Concentrated Water Soluble Fraction of Milk as a Source of Vitamin B. G. C. Supplee, Odessa D. Dow, and G. E. Flanigan.....	420

## NOVEMBER. No. 6

The Effect of Lecithin in Dairy Products upon Butter Fat Determinations. O. W. Chapman.....	429
The Effect of Distilled Water upon the Tendency to Colony Formation upon Petri Plates. C. S. Mudge and B. M. Lawler.....	436
Distribution and Growth of Bacteria in Butter. Otto Rahn and H. H. Boysen.....	446
Heat Coagulation of Evaporated Milk as Affected by Mixing Different Grades of Raw Milk. Byron H. Webb.....	471
The Relation of Soybean Hay and Ground Soybeans to Flavor and Composi- tion of Milk and Butter. W. B. Nevens and P. H. Tracy.....	479
Cottonseed Meal as a Feed for Dairy Calves. O. E. Reed, C. F. Huffman, and L. H. Addington.....	488
Some Observations on the Bacterial Content of Dried Milk. H. Macy.....	516
Proceedings of the Annual Meeting of the American Dairy Science Association.....	527
Index.....	533





# THE EFFECT OF THE AGES OF SIRE AND DAM ON THE AVERAGE BUTTERFAT PRODUCTION OF OFF-SPRING IN DAIRY CATTLE\*

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Since early times many breeders, from their observations of dairy cattle, have concluded that immaturity and senescence of parents have a marked effect upon the milk and butterfat production of the progeny. Considering the economic importance of the effects of these factors upon the dairy industry, there has been a very scant amount of analytical consideration given to determine any variations in the performance records of progeny due to different ages of parents.

It is well known that the age of parents affect fecundity, and birth weights in domesticated animals, and many investigators believe that sex-ratios are also affected by the same influences. Carmichael and Rice (3) in their study of fecundity in swine found that there was a gradual increase in the size of the litter as the sows grew older up to the time they were three years of age, after which the tendency for decrease in litter size became apparent. Jones and Rouse (10) conclude that in multiparous animals the size of the litters and the frequency of production of litters increase with the age of the female to a maximum and then a decline follows. They also give evidence that in general there is an increase of litter size with the increasing age of the male. The observations made on the fecundity of uniparous animals coincide with similar ones on multiparous animals. The conclusions are confirmed by King (11), Marshall (13), Frolich and Georgs (8), Heape (9), Duncan (6) and others.

Donaldson (5) reports that the birth weight of rats increases with the increasing age of the dam. Kopec (12), as cited by the Experiment Station Record, in a statistical study on weights of young and size of litter in rabbits found that the age of dams did

\* Received for publication August 1, 1927.

not influence the size of litters or birth weight during the first two years, but that dams three years old produced larger litters and heavier young. Eckles (7) notes that the maturity of the dairy cow has some effect upon weights of calves at time of birth. The average weight of the first born is less than the subsequent calves, and there is a tendency for cows of an advanced age to produce calves rather smaller than those from cows in the prime of life.

Parkes (15), in his studies of mammalian sex-ratios, concludes that there is much contradiction in the results of the many investigations determining the effect of the age of the male upon sex-ratios, but found there was much evidence that young mothers have an excess of males and that the male percentage decreases with the advancing age of the dams.

Redfield (18) as quoted by Marshall (14) published a dynamic theory of development in which he assumed that by exercise a horse acquired "dynamic development" which facilitated speed and which was transmitted to his offspring. His data, collected from the Index Digest for trotting horses, show that the average age of sires of 2:10 trotters was more than nine years at the time they were born. Marshall, in a similar study, believes that the records do not reveal any superiority of old sires over the younger ones.

Chaudhuri (4) studied the relation of the age of the sires and dams of the prize winners in the Shorthorn classes at the Highland and Agricultural Society's Shows and found that with both the sires and dams there was a tendency for a greater number of offspring from immature animals but attributed these tendencies to the fact that young animals are kept in greater numbers by breeders in Scotland for breeding purposes. Allen (1) determined the ages of the sires and dams for cows that made very high and very low seven-day records as reported by Volume 27 of the Advanced Registry year book published by the Holstein-Friesian Association of America and concluded from the study of these extreme quartiles of production that the parentage of the high producing dairy cows is no older than the parentage of comparatively low producing cows.

The amount of data presented in the literature dealing with the relationship of age of parents and quality of offspring are very meager, although from the practical breeding viewpoint it is clearly advantageous to know definitely whether dairy heifers born from immature or aged parents will on the average produce as much butterfat annually as those born from parents during the more active period of maturity. To get more quantitative expressions on this question the writers made a study of the records of Guernsey cows as reported in the Advanced Registry of the American Guernsey Cattle Club, to January 1, 1924, and have determined the relation between the age of the parents and butterfat production in the offspring.

#### PROCEDURE

Butterfat records, and dates of birth were secured for the daughters of all the sires of the Guernsey breed that have ten or more Advanced Registry daughters, making this special selection of sires because it was believed that the selection made by breeders in discarding inferior bulls would be partially eliminated, and that such a selection would decrease the average production of groups sired by inferior animals that would fall primarily in young groups and not in older ones.

Since it has been pointed out by Pearl and Patterson (17), Pearl, Gowen and Miner (16), and Brody, Ragsdale, and Turner (2), that fat production in dairy cattle gradually increases and decreases in a logarithmic form, rising at an ever decreasing rate until the age of maximum production is reached and then gradually decreasing at an even decreasing rate with the onset of old age, it was deemed necessary to change the records of cows made at different ages to a common age record in order to secure comparative values from all records studied. All records were changed to a mature equivalent record by multiplying the annual fat record by the different age conversion factors given in table 1 as used by Turner (19). The term fat record throughout the remaining discussion will indicate a mature or mature equivalent record. In case a cow had more than one Advanced Registry record, the largest mature equivalent record was used.

When the fat records were determined and the date of birth recorded, the ages of the sires and dams were computed at the time of birth. The fat records were then grouped according to the age of parents at the time the progeny was born as shown in the correlation surfaces (tables 2 and 3). The age intervals include all animals within six months of the age given.

TABLE 1  
*Age conversion factor for Guernsey cows*

AGE	FACTOR
years	
Under 2	
2- 2½	1.313
2½- 3	1.251
3- 3½	1.194
3½- 4	1.142
4- 4½	1.100
4½- 5	1.064
5- 5½	1.041
5½- 6	1.023
6- 6½	1.013
6½- 7	1.006
7- 7½	1.000
7½- 8	1.000
8- 8½	1.004
8½- 9	1.009
9- 9½	1.017
9½-10	1.029
10-10½	1.041
10½-11	1.058
11-11½	1.075
11½-12	1.093
12-12½	1.113
12½-13	1.137
13-13½	1.162
13½-14	1.191
14-14½	1.219

From the same data, all fat records were secured that had been made by the progeny of parents that were of the same age at the time they were mated, in order that any age effect a parent might exert upon the progeny would not be counter-balanced

# BUTTERFAT PRODUCTION IN DAIRY CATTLE

## TABLE 2

*Correlation surface for age of Guernsey cows and yearly butterfat production of daughters*

CLASS		AGE OF DAMS IN YEARS															TOTAL
		2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Daughters' yearly fat production in pounds	375	35	46	38	30	37	17	15	12	4	2	5	3	2	1	247	
	425	62	81	75	60	48	37	31	21	17	8	4	5	4	5	488	
	475	74	87	72	84	61	47	37	35	22	16	10	5	4	1	555	
	525	64	90	105	69	62	52	42	26	15	13	13	6	8	7	572	
	575	54	66	60	85	62	54	32	26	18	17	13	5	1	3	496	
	625	42	49	64	60	57	44	30	29	19	13	3	5	5	4	424	
	675	34	55	43	43	45	43	24	16	12	9	6	2	3	3	338	
	725	33	34	39	33	22	26	18	9	6	2	7	4	0	0	233	
	775	12	23	22	21	15	16	9	7	2	5	2	0	1	0	135	
	825	7	15	5	14	9	8	6	5	4	6	2	1	1	0	83	
	875	5	7	3	8	10	2	2	2	1	3	0	0	0	2	45	
	925	1	2	4	6	4	4	1	0	0	0	0	0	0	0	22	
975	0	3	2	3	0	1	1	0	0	0	0	0	0	0	10		
1025	0	1	1	1	1	0	0	1	2	0	0	0	0	0	7		
Total.....		423	559	533	517	433	351	248	189	122	94	65	36	29	26	3,625	

## TABLE 3

*Correlation surface for age of Guernsey sires and yearly butterfat production of daughters*

CLASS		AGE OF SIRE IN YEARS															TOTAL
		2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Daughters' yearly fat productions in pounds	375	45	78	60	55	20	17	8	8	11	2	5	3	1	0	313	
	425	65	138	120	89	52	31	23	17	11	5	6	3	0	0	560	
	475	65	175	130	86	69	27	26	26	19	15	6	2	3	1	651	
	525	64	153	137	101	63	37	26	24	12	11	4	3	2	2	639	
	575	61	117	123	76	47	48	30	23	8	5	11	3	1	1	554	
	625	47	112	107	61	44	44	19	22	12	8	0	6	2	0	484	
	675	34	79	47	56	33	33	33	18	1	6	2	3	1	1	347	
	725	31	54	47	23	28	20	18	7	4	2	0	3	3	0	240	
	775	18	20	27	17	18	11	8	6	6	2	2	1	2	1	139	
	825	11	17	12	12	7	6	8	4	0	1	1	1	0	0	80	
	875	5	9	6	6	5	3	4	2	3	1	0	0	0	0	44	
	925	2	2	4	7	2	1	1	1	0	0	0	0	1	0	21	
	975	0	3	2	2	2	0	1	0	0	1	0	0	0	0	11	
	1075	1	0	1	1	2	2	0	0	0	0	0	0	0	0	7	
Total.....		449	957	823	592	392	280	205	159	87	61	37	28	16	6	4,090	

by the effect of an older or younger age of the other parent. These data are presented in the correlation surface (table 4).

The coefficients of correlation for these three groups of data are presented in table 5.

The coefficients of correlation representing the relationship

TABLE 4

*Correlation surface for Guernsey sires and dams of same age and annual butterfat production of daughters*

CLASS	AGE OF BOTH SIRE AND DAM IN YEARS											TOTAL
	2	3	4	5	6	7	8	9	10	11	12	
Daughters' yearly butterfat production in pounds												
375	15	18	7	9	3	1	0	0	0	0	0	53
425	22	21	17	11	6	4	2	0	1	0	0	84
475	33	15	19	12	3	0	6	1	0	3	0	92
525	30	20	16	10	4	3	2	0	0	0	0	85
575	23	11	12	6	4	4	3	0	1	0	1	65
625	17	16	12	6	7	3	2	3	0	0	0	65
675	15	11	8	5	5	5	1	0	2	0	0	52
725	13	11	4	4	5	3	1	0	0	0	0	41
775	5	6	2	1	0	1	0	0	0	0	0	15
825	3	2	2	2	0	0	0	0	0	0	0	9
875	0	1	0	2	0	0	0	0	0	0	0	3
925	0	2	1	3	0	0	0	0	0	0	0	6
975	0	1	0	0	0	0	0	0	0	0	0	1
1025	0	0	0	1	0	0	0	0	0	0	0	1
Total.....	176	134	100	72	37	24	17	4	4	3	1	572

TABLE 5

*Coefficients of correlation between annual butterfat production of Guernsey cows and age of parents at time of birth*

	COEFFICIENT OF CORRELATION
Age of sires and butterfat records of progeny.....	0.070 $\pm$ 0.010
Age of dams and butterfat records of progeny.....	0.021 $\pm$ 0.011
Age of sire and dam (same age) and butterfat records of progeny.	0.050 $\pm$ 0.028

between the annual fat production of these cows and the age of parents at time of birth cannot be considered as significant positive correlations in any of the three groups. The only indication of an increase in annual fat production with age of parents

is in the case of the very slight relationship between the age of the sires and the progeny records.

In order to observe readily any groups of variates by a certain age parents, either sire or dam, that diverge from the average mean of production for the total group, the arithmetical averages were obtained for the different age groups of dams and sires. These results are plotted in figure 1. According to this figure, there is not a significant indication that any particular age of dams have a pronounced effect upon the records of the daughters. There is a tendency, however, for the average records of the

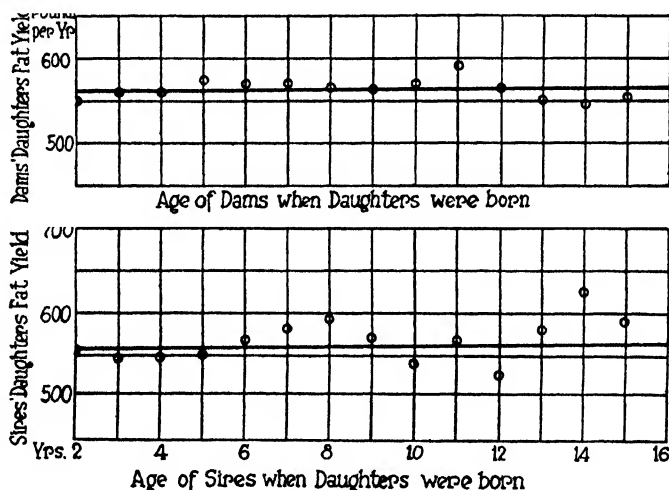


FIG. 1. AVERAGE BUTTERFAT PRODUCTION OF DAUGHTERS OF SIRES AND DAMS AT DIFFERENT AGES

daughters of the sires in the seven and eight-year-old groups to increase, but as the age increases the groups fall around the mean for the entire mass of variates.

The data presented indicate that on the average, the ability of the parent to implant the characters for high butterfat production in its offspring does not increase with maturity nor decrease with senescence. In other words, the age of the sire and dam does not affect the butterfat production of the progeny in the same manner that it does birth weights, fecundity and



sex-ratios in animals. Butterfat production, like sex-ratios and all similar characters, is an inherited character governed by genetic continuity but is not disturbed by any physiological change in the parents' bodies due to immaturity or senescence.

#### REFERENCES

- (1) ALLEN, C. L.: The effect of the age of the sire and dam on the quality of offspring in dairy cows. *Jour. Heredity*, 1922, xiii.
- (2) BRODY, S., RAGSDALE, A. C., AND TURNER, C. W.: The rate of growth and senescence as measured by the rise of milk secretion with age. *Jour. Gen. Phys.*, 1923, vi, no. 1.
- (3) CARMICHAEL, W. J., AND RICE, JOHN B.: Variations in farrow. *Ill. Bul.* 226, 1920.
- (4) CHAUDHURI, A. C.: Age and quality of offspring. *Jour. Heredity*, 1926, xvii.
- (5) DONALDSON, H. H.: The rat. *Memoirs of the Wistar Inst. Anat. and Biol.* No. 6, Philadelphia, 1915.
- (6) DUNCAN, J. MATHEWS: Fecundity, Fertility, Sterility and Allied Topics. Adam and Charles Black, Edinburgh, Scotland.
- (7) ECKLES, C. H.: Dairy Cattle and Milk Production. The Macmillan Co.
- (8) FROLICH AND GEORGS: Fruchtbarkeit und Geschlechtsverhältnis beim weissen Edelschwein. *Jahrbuch für Wissenschaftliche und praktische Tiergucht*, 6. Jahrg., 1911, Hannover.
- (9) HEAPE, H.: Fertility in sheep. *Jour. Royal Agricultural Society of England*, 3rd Series, 1899.
- (10) JONES, S. V. H., AND ROUSE, J. E.: The relation of the age of dam to observed fecundity in domesticated animals. *Jour. Dairy Sci.*, 1920, iii.
- (11) KING, HELEN DEAN: The relation of age to fertility in the rat. *Anatomical Record*, 1916, xi, no. 5.
- (12) KOPEC, S.: The influence of certain factors on the weight of the newborn of mammals (Trans. title). (Pam. Paust. Inst. Nauk. Gosp. Wiekaj, Pulawach.) (Mem. Inst. Nat'l. Polon, Econ. Rulale Pulawy, 4, 1923.)
- (13) MARSHALL, F. H. A.: The Physiology of Reproduction. Longman, Green and Co., 1922.
- (14) MARSHALL, F. R.: Age of trotting horse sires. *The Amer. Naturalist*, 1909, xliii.
- (15) PARKES, A. S.: The mammalian sex-ratios. *Biological Rev. and Biological Proceedings of the Cambridge Philosophical Society*, vol. ii, no. 1, 1926.
- (16) PEARL, R., GOWEN, J. W., AND MINER, J. R.: Studies in milk secretion. VII. *Me. Agr. Exp. Sta. Bul.* 281, 1919.
- (17) PEARL, R., AND PATTERSON, S. W.: The change of milk flow with age as determined from seven-day records of Jersey cows. *Me. Agr. Exp. Sta. Bul.* 262, 1917.
- (18) REDFIELD, C. L.: *Horse World*, February 27, 1906.
- (19) TURNER, C. W.: A comparison of Guernsey sires. *Mo. Agr. Exp. Sta. Res. Bul.* 79, 1925.

## THE FREEZING POINT OF CHEDDAR CHEESE: INJURY OF CHEESE BY FREEZING\*

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The freezing point of cheese and the injury of cheese by freezing are of vital importance in the determination of freight rates and the adjustment of claims for damage due to freezing. In response to a request for information on this subject for use in connection with a freight rate hearing before the Interstate Commerce Commission a search of the literature was made. No published work could be found on the freezing point of cheese, and the only work found that approached the subject of injury by freezing, was the work by Babcock and Russell (1, 2) on the cold curing of cheese.

Watson and Leighton (3) in response to a similar request, report the freezing point of a Cheddar cheese as  $-12.9^{\circ}\text{C}$ . and of a processed Cheddar cheese as  $-6.9^{\circ}\text{C}$ ., and call attention that the freezing point of the same kind of cheese will vary depending upon the moisture content and degree of ripening.

Babcock and Russell in their study of the cold curing of cheese, stored some Cheddar cheese directly from the press at  $15^{\circ}\text{F}$ ., for various lengths of time. They found that cheese stored at  $15^{\circ}\text{F}$ . for seven months had a score of 38 on flavor and 24 on texture. This cheese, however, when subsequently stored at  $40^{\circ}\text{F}$ . increased in score to 42 on flavor and 28 on texture. (In the score card used, 45 on flavor and 30 on texture were perfect.) Cheese stored at  $15^{\circ}\text{F}$ . for five months, then removed to  $40^{\circ}\text{F}$ . for seven months, scored 44 on flavor and 28 on texture. Contrary to the then and still prevailing impression, subjecting the cheese to this low temperature, failed to develop a bitter flavor in the cheese.

Because of the paucity of information to be found on this subject, a limited amount of experimental work was undertaken to

\* Published with the permission of the Director of the Wisconsin Agricultural Experiment Station. Received for publication June 6, 1927.

meet the request. The results of these experiments here reported must be regarded as a preliminary report; more extended experiments on Cheddar and other varieties of cheese are being undertaken.

#### THE FREEZING POINT OF CHEDDAR CHEESE

##### *Method used*

At the outset freezing point determinations were made using thermocouples of copper-constantin junctions. It was soon found that the freezing points of different samples of Cheddar cheese differed widely, and in view of these wide differences and other considerations, an accuracy of  $0.1^{\circ}\text{C}.$  was considered ample. With no greater precision required, and with thermometers of the desired precision readily available, it was decided to use thermometers rather than thermocouples which require vigilant care to obtain trustworthy results.

The thermometer used in this work was a mercury thermometer calibrated to  $0.1^{\circ}\text{C}.$  The bulb of the thermometer was 10 mm. long and tapered from a diameter of 5 mm. at the upper part to 2.5 mm. at the tip. In the first determinations 2-inch cubes of cheese were used. The thermometer bulb was inserted to approximately the center of this cube after a suitable sized hole had been cut with a cork borer. The cube of cheese with the thermometer was placed in the ice cream hardening room which ranged in temperature from  $-18^{\circ}$  to  $-26^{\circ}\text{C}.$  Temperature readings of the cheese were made at suitable intervals and recorded.

With the 2-inch cubes of cheese, the typical freezing curve was not obtainable with certainty, in that supercooling usually did not occur. It was thought that this was due to the size of the cube; supercooling and freezing would take place at the surface before the center of the cheese cube had reached the freezing point; the ice crystals thus formed in the outer parts of the cube would then inoculate the crystallization as the cooling proceeded towards the center, and thus prevent supercooling of the cheese immediately surrounding the thermometer bulb. In order to

obtain a characteristic freezing point curve with greater certainty, it was, therefore, decided to use a 1-inch cube of cheese. No further trouble was then experienced, characteristic supercooling was obtained in all cases where the 1-inch cube was used.

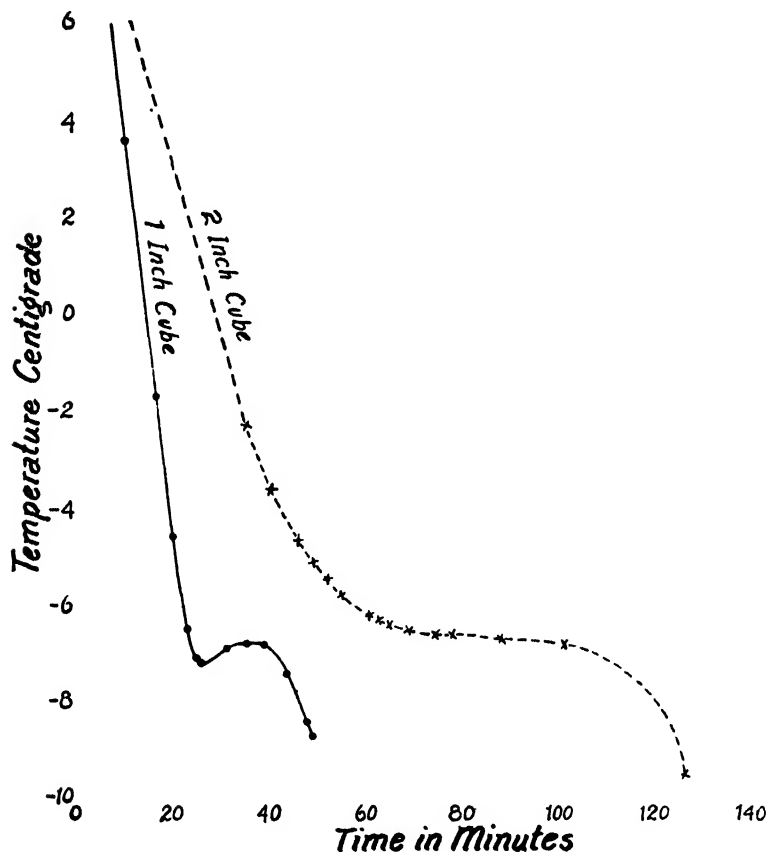


FIG. 1

Figure 1 shows the results obtained with a 1-inch and a 2-inch cube of the same cheese. The highest point to which the temperature rose after supercooling in the 1-inch cube was  $-6.8^{\circ}\text{C}$ . In the case of the 2-inch cube there was no supercooling, but the temperature curve indicated that the freezing point was  $-6.65^{\circ}\text{C}$ .

(The results in figure 1 correspond to Cheese No. 7 in table 1.) The agreement between these two results is considered close enough to justify the inclusion of the results obtained with the 2-inch cubes. The agreement is a fair indication of the degree of accuracy in the results reported.

TABLE 1  
*The freezing point of Cheddar cheese*

CHEESE NUMBER	AGE OF THE CHEESE DAYS	MOISTURE	SIZE OF CHEESE CUBE USED	TEMPERATURE OF FREEZING ROOM	FREEZING POINT
		<i>per cent</i>		<i>°F.</i>	<i>°C.</i>
1	55		2	0	-5.55
2	35	33.19	2	+6	-5.75
3	76	36.69	2	-4	-6.4
4	23	35.12	2	0	-5.5
5	42	36.37	2	-16	-5.6
6	381	33.77	2	-16	-4.3
7	74	33.41	1	-12	-14.3
			1	-10	-6.8
			2	-10	-6.65
8	112	36.03	1	-8	-5.62
			1	-8	-5.50
9	110	35.02	1	-8	-6.15
			1	-8	-6.40*
10	41	37.10	1	-8	-5.15*
			1	-8	-4.80
11	39	37.93	1	-8	-5.40*
			1	-8	-5.15
12	389	32.06	1	+6	-11.3
13	347	33.17	1	+6	-10.8
14	394	32.80	1	+6	-12.9
15	346	33.34	1	+6	-10.9

\* Excessive supercooling.

Greater accuracy than the above was considered unnecessary because (1) there is a much wider difference in the freezing points of different samples of the same variety of cheese; (2) the practical application of these results would probably not take cognizance of fractions of a degree; (3) in duplicate determinations on the same cheese wider differences are likely to be found because of non-uniformity of the cheese; (4) in determining the freezing

point of a solid it is useless to strive for the same accuracy that can be attained in the case of liquids because stirring is impossible and it is difficult to control the extent of supercooling.

### *Results obtained*

The results obtained in the work on Cheddar cheese are given in table 1. The size of the cheese cubes used in each case and the temperature of the room in which the freezing took place are given. The age and the moisture content of the cheese are also given because of their relation to the freezing point.

An inspection of this table shows that the freezing points of the Cheddar cheese samples tested ranged from  $-4.3^{\circ}$  to  $-14.3^{\circ}\text{C}$ . In seeking the cause for this wide difference, it will be noted that the lowest freezing point was obtained with the cheese that was over a year old. (Sample No. 6 and No. 12 to 15 inclusive, table 1.) The indication is that the age of the cheese, the degree of ripeness, is an important factor in the freezing point.

On the basis of theoretical knowledge, we know that the freezing point of cheese is dependent upon the amount of water present and the amount and character of the substances dissolved in this water. Thus the water content and the salt content of the cheese must be factors that fix the freezing point of cheese. In addition water soluble substances other than salt also must be a factor. It is known that in the cheese ripening, the water soluble substances increase; thus the degree of ripeness of the cheese must also be a factor. The results thus far obtained indicate that this latter factor is of such importance that it overshadows the importance of the moisture and salt content of the cheese within the limits of variation encountered in these samples.

### THE INJURY OF CHEESE BY FREEZING

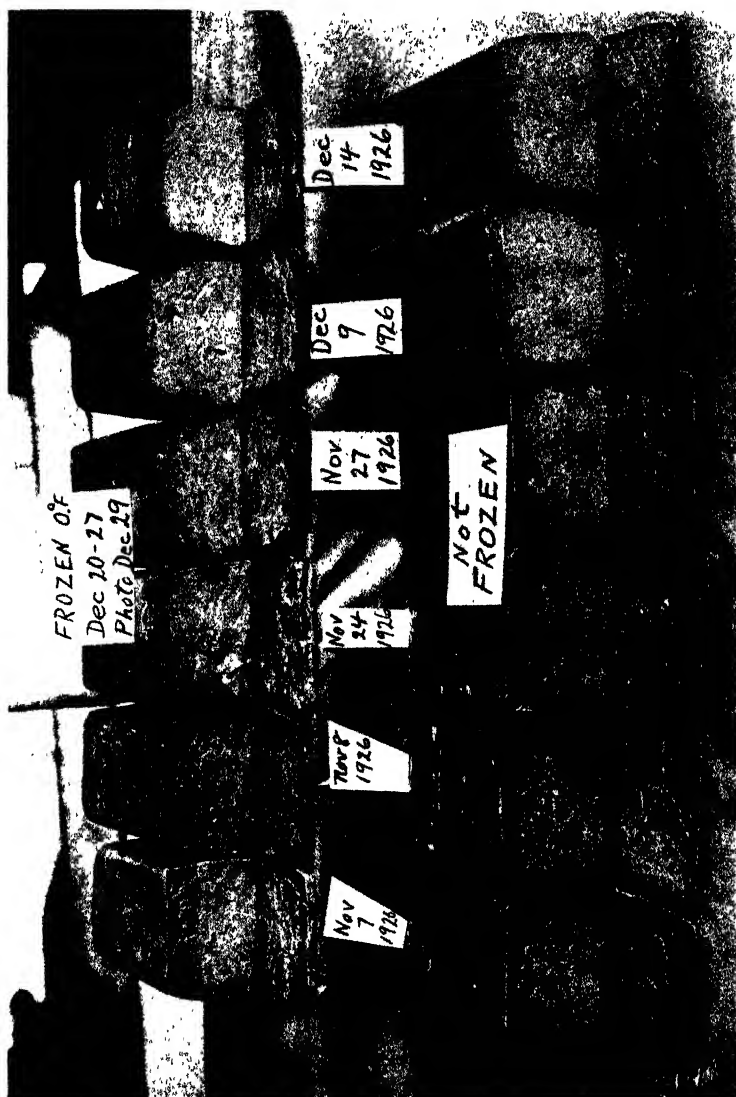
In this preliminary study of the injury of cheese by freezing, a limited number of cheeses were subjected to temperatures well below the freezing point of cheese for various lengths of time. The cheeses were subsequently stored in the cheese curing room to thaw out gradually and were then examined. In the case of six different cheeses the scores of the frozen and unfrozen cheeses

and a photograph showing the appearance of these cheeses, are available. These cheeses had been kept in the ice cream hardening room for seven days, the temperature ranging from 0° to -10°F. Two days after the cheeses had been taken out of the cold room and placed in the cheese curing room to thaw out, they

TABLE 2  
*The scores for flavor and texture of frozen and unfrozen cheeses*

CHEDDAR CHEESE OF	JUDGE NUMBER	SCORE FOR FLAVOR		SCORE FOR TEXTURE	
		Frozen	Unfrozen	Frozen	Unfrozen
November 7.....	1	24	24	35	36
	2	26	26	36	36
	3	23	24	34	35
November 8.....	1	24	24	35	36
	2	26	26	35	35
	3	24	24½	36	36
November 24.....	1	25	25	34	36
	2	26	26	34	34
	3	25½	26	34	35½
November 27.....	1	23	23	35	36
	2	25	25	34	35
	3	23	23	35	36
December 9.....	1	25	26	35	36
	2	26	26	36	37
	3	26½	26	34	34½
December 14.....	1	24	24	36	36
	2	26	26	34	34
	3	27	26	34	35
Average.....		24.94	25.03	34.79	35.50

were examined and photographed, and a month later they were scored by three cheese judges. In each case a corresponding cheese made from the same vat of milk was used as a control. The results of the scorings are summarized in table 2, and figure 2 is a photograph of these cheeses, showing the appearance of the cut surfaces.





It will be noted that the difference in flavor as found by the three judges is so slight as to be negligible. The judges comments are not reproduced here, but it should be noted here that in no case did the judges indicate a bitter flavor in the frozen cheese as compared with the corresponding unfrozen cheese.

The results of the scorings show that the freezing injury is confined primarily to the texture, which becomes more or less crumbly on freezing. Figure 2 records this tendency to crumble in a fairly satisfactory manner. The tendency of freezing to disrupt the texture of the cheese is most evident at the sutures where the curd particles fused together in the pressing of the cheese. Note that the lighter lines at the sutures are decidedly more pronounced in the frozen cheeses than in the corresponding unfrozen cheeses.

Observations made on these six and other cheeses that were frozen showed that in freezing paraffined cheese, the paraffin layer flaked off from the cheese to such an extent that reparaftining would be advisable.

Examination of the frozen cheeses also gave the following additional indications:

1. The damage to the texture by freezing is dependent upon the texture and make-up of the cheese. Cheese in which the sutures were well knit and in which there were few or no mechanical holes, showed less injury to the texture than cheeses not so perfectly made.

2. After the cheeses had been frozen, storing them at normal cheese storage temperatures accomplished a gradual improvement in the texture of the cheese. In some cases this improvement was such that after four weeks it was difficult or practically impossible to distinguish between frozen and the corresponding unfrozen cheese. This recovery in the texture of the cheese observed here is in harmony with the results of Babcock and Russell cited above.

3. After such recovery of the texture, distinction between frozen and unfrozen cheese could be made by observing the manner in which the cheese checked on drying at the surface, the frozen cheese checking more than the corresponding unfrozen cheese.

The above indications were sufficiently evident to warrant their mention here, but they cannot be stated as unqualified conclusions until further substantiated by more extended experiments. In the work planned on the basis of the preliminary results, observations on the various points mentioned in this paper are to be included.

#### SUMMARY

1. The freezing point of cheese can be determined with sufficient accuracy by the use of a 1-inch cube of cheese and a suitable thermometer.

2. The freezing points found for 15 different Cheddar cheeses ranged from  $-4.3^{\circ}$  to  $-14.3^{\circ}\text{C}$ .

3. In addition to the moisture and salt content, the age of the cheese is an important factor in the freezing point.

4. The cheese subjected to freezing showed no perceptible injury to the flavor.

5. Freezing caused the texture of the cheese to be crumbly. The extent of crumbliness developed was dependent upon the texture and make-up of the cheese before freezing.

6. Freezing caused the paraffin to flake off from paraffined cheese.

7. On storage at favorable temperatures after freezing, the cheese texture recovered, in some cases apparently completely.

8. After the texture had recovered, distinction between frozen and unfrozen cheese could be made on the basis of the manner in which the cheese checked on drying at the surface.

The author wishes to acknowledge the interest and assistance in this work by J. L. Sammis, H. L. Templeton, P. E. McNall, A. T. Bruhn, W. Winder, and Math. Michels.

#### REFERENCES

- (1) BARCOCK, S. M., AND RUSSELL, H. L.: Wis. Ag. Exp. Sta. Bul. 94.
- (2) BARCOCK, S. M., AND RUSSELL, H. L.: Wis. Ag. Exp. Sta., 18th An. Rep., pp. 136-162.
- (3) WATSON, PAUL D., AND LEIGHTON, ALAN: Private communication.

# A STUDY OF THE YELLOW COCCI WHICH SURVIVE PASTEURIZATION\*

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## INTRODUCTION

In investigations at the Iowa Agricultural Experiment Station on the bacteria present in various dairy products, the plates frequently showed yellow colonies, sometimes in considerable numbers. Both the surface and subsurface colonies were usually comparatively large and the color quite intense so that they were very conspicuous. These colonies seemed to be especially numerous on plates poured with pasteurized cream or butter made from it, and because they made up such a large part of the flora with these materials a study of the organisms producing them was undertaken. The resistance to heat, the variations that occur and the general action on milk were given the most attention; the results obtained are reported herein.

## HISTORICAL

Cocci producing yellow colonies have been isolated by a number of investigators in the study and classification of the organisms in various materials. Certain of these seem to be common in air and water, from which they could easily find their way into milk. Bergey<sup>1</sup> recognized a number of species, some of which have been isolated from dairy products. Various investigations<sup>2</sup> have shown that micrococci producing yellow colonies on agar are rather common among the organisms coming from the interior of the udder.

Marshall<sup>3</sup> isolated a rod shaped organism that produced a

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<sup>1</sup> Manual of Determinative Bacteriology, 2nd edition, 1925.

<sup>2</sup> N. Y. Agr. Expt. Sta., Tech. Bul. 27. 1913.

<sup>3</sup> Mich. State Agr. Col. Expt. Sta., Bul. 147, 1897.

lemon yellow pigment from pasteurized milk but found no cocci among 19 surviving organisms. Russell and Hastings<sup>4</sup> studied the influence of the formation of a surface membrane on the resistance of the organisms in milk and used a micrococcus which retained its vitality at temperatures considerably above those usually used for pasteurization. Conn and Esten<sup>5</sup> found that nearly all of the yellow colony producing bacteria present in milk in the vicinity of Storrs were sarcinae; they state that this group, although nearly always present in small numbers in milk, almost universally disappears after a few hours and never multiplies to any extent. Ayers and Johnson<sup>6</sup> found various types of cocci surviving pasteurization and the same thing has been noted by other investigators. Brannon and Prucha,<sup>7</sup> in studies on the resistance of organisms to pasteurization, worked with a micrococcus which, when it was isolated, was causing counts of a million or more in pasteurized milk. They also found that *Sarcina lutea* was not destroyed by pasteurization.

#### EXPERIMENTAL

Preliminary observations showed that practically all of the organisms producing yellow colonies on the plates were cocci and accordingly only this morphological type was considered in the studies carried out.

A total of 113 cultures were used, most of which came from dairy products; 51 were from butter made from pasteurized sweet cream, 4 from raw cream, 28 from pasteurized cream, 11 from raw milk, 7 from pasteurized milk, 3 from boiled milk, 1 from starter, 4 from churn rinsings and 4 from air. The numbers of yellow colonies on plates poured with different samples of dairy products varied a great deal, but were commonly large enough so that the organisms very evidently were present in the materials plated and were not the result of air contamination; moreover the plates representing different dilutions showed proportionate num-

<sup>4</sup> Univ. of Wis., Agr. Expt. Sta., 18th An. Rpt., 1901. p. 185.

<sup>5</sup> Storrs Agr. Expt. Sta., 16th An. Rpt., 1904, p. 27.

<sup>6</sup> U. S. Dept. Agr., B. A. I., Bul. 161, 1913.

<sup>7</sup> Jr. Dairy Science, 1927, 10, p. 263.

bers. All of the cultures were purified by plating at least once and often several times.

*Resistance of the organisms to heat.* In order to establish the ability of the organisms to survive pasteurization exposures, the heat resistance was studied using 12 cultures from various sources and representing somewhat different types. The heating was carried out as follows: Test tubes containing the various materials and stoppered with cotton were put in a large water bath so that the water was well above the surface of the medium and heated to the desired temperature. The tubes were then inoculated, held for thirty minutes and plunged into cold water so that they would cool quickly. In case of question as to whether or not growth had occurred, transfers were made to agar slopes. There was no evidence of a scum at the surface of the material being heated in any of the trials, due undoubtedly to the limiting of evaporation by the cotton stoppers. This method of heating was selected because it was considered to give results more comparable to heating in a vat than would sealed tubes; in a large number of attempts the use of the method resulted in the destruction of various streptococci that were known to be destroyed by pasteurization.

When heated in whole milk all of the cultures grew after an exposure to 70°C. for thirty minutes; 2 of them failed to grow after heating to 75°C. for thirty minutes, while with 2 others the growth was questionable, and after heating to 80°C. for thirty minutes, none of them grew. In lactose bouillon all of the cultures grew after an exposure to 75°C. for thirty minutes while 2 survived 80°C. for thirty minutes. In table cream containing approximately 20 per cent fat, 11 of the cultures survived 70°C., 10 survived 75°C., and 2 survived 80°C., the time being thirty minutes in all cases.

The above results show that the organisms can resist the usual pasteurization exposures, and that, although there were some variations in the resistance of the organisms to heat in the different materials, these were not pronounced. The same general resistance to the usual pasteurization exposures was secured in litmus skimmilk, sucrose bouillon, and ice cream mix.

The influence of the acid content of milk on the heat resistance of the organisms was studied using skimmilk of normal acidity and another portion of the same lot in which *Streptococcus lactis* had produced sufficient acid to cause coagulation. In the normal milk all 12 of the organisms survived 75°C. for thirty minutes, while in the acid milk only one culture survived 65°C. for thirty minutes and all were killed at 70°C. for thirty minutes. In the presence of acid the heat seems to be considerably more destructive than in its absence. Similar results have been obtained in this laboratory in studies on the resistance of *S. lactis* to heat.

The decreased resistance of organisms to pasteurization exposures in the presence of acid is undoubtedly of importance in the pasteurization of sour cream. It may explain in part the greater frequency with which the cocci producing yellow colonies are encountered in plates from sweet cream than from sour cream. It may also account for the higher bacterial efficiencies obtained in the pasteurization of sour cream, although the comparatively low heat resistance of many of the *S. lactis* organisms, which make up most of the flora, is a factor here also.

#### GENERAL DESCRIPTION OF THE ORGANISMS STUDIED

The colonies of the yellow cocci on whey agar plates were first of a pale yellow color which later deepened to a lemon or sulphur yellow. To the naked eye the surface colonies appeared round, slightly raised, entire, and glistening and under the low power of the microscope they were granular with an entire edge. In microscopic preparations the cells were spherical, of medium size, and arranged singly, in pairs, in tetrads or sometimes in packets. The organisms took the ordinary stains quite readily and were gram positive although gram negative cells were not uncommon, especially in old cultures. They grew well in liquid or on solid media, but brought about changes in milk only very slowly. Gelatine liquefaction and milk digestion occurred with some of the cultures. Acid production in milk and bouillons, when it took place at all, was slight. Growth was best under aerobic conditions, and was rapid at both 21° and 37°C.

## DIVISION OF THE ORGANISMS STUDIED

Various characters, especially the morphology, the liquefaction of gelatin, the action on litmus milk, and the growth on slopes suggested a division into three types—A, B, and C.

*Distinguishing features of Type A.* Type A may be characterized as follows: It was a fairly large coccus, usually arranged in tetrads, and under favorable conditions in packets. It grew well at both room temperature and 37°C. Litmus milk did not materially change in appearance except for a whitish-yellow precipitate in the bottoms of the tubes. Gelatine was slowly liquefied. Growth on whey agar slopes was filiform, smooth edged, slightly raised, opaque, and of a distinct lemon color.

*Distinguishing features of Type B.* Type B may be characterized as follows: It was a medium sized coccus, occurring singly, in pairs, or in tetrads. The organisms first reddened litmus milk and reduced it and then coagulated and slowly digested it. Gelatine was liquefied very slowly. The growth on whey agar slopes was filiform, opaque, slightly raised, and of a lemon yellow color.

*Distinguishing features of Type C.* Type C may be characterized as follows: The cells were arranged singly or in pairs and there was considerable variation in size. Acid was produced slowly in litmus milk, and gelatine was rarely liquefied. The growth on whey agar slopes was filiform, opaque, raised, glistening and from a pale to a lemon yellow color.

The descriptions of the yellow cocci that have been published do not afford a clear cut classification of the organisms that were studied. This is partly due to the fact that there are differences in the descriptions of the same organism by different authors. A consideration of the various classifications studied, especially those of Bergey and Hucker<sup>1</sup> suggest that Type A should be considered as *Sarcina lutea*, Type B as *Micrococcus varians*, and type C as *Micrococcus luteus*.

The important point seems to be that there are variations in the heat resistant yellow cocci that are found so regularly in dairy products, especially those subjected to pasteurization.

<sup>1</sup> N. Y. Agr. Exp. Sta., Tech. Bul. 102, 1924.

Apparently resistance to heat is a rather common character of a group of organisms that also has the general property of producing yellow colonies.

#### GENERAL ACTION OF ORGANISMS IN MILK

The organisms brought about various types of changes in milk but these occurred comparatively slowly so that these organisms are probably of little importance from the standpoint of deterioration of milk or cream under practical conditions. The comparative resistance of the organisms to unfavorable conditions is illustrated by the growth on transfer of agar cultures that had been held nine months in a cooler at from 0° to 10°C. and is also evident from their presence in air. It would accordingly be expected that the yellow cocci might persist in various materials, including dairy products, for extended periods.

#### SUMMARY AND CONCLUSIONS

On plates poured with dairy products, especially those that had been subjected to heat, there were often yellow colonies of cocci. In general these organisms resisted the usual pasteurization exposures for market milk; they were resistant in skimmilk, whole milk, cream, bouillon and ice cream mix. The organisms were less resistant in milk containing sufficient acid to cause coagulation than in normal milk.

The yellow cocci found in dairy products were of several types. They produced changes in milk or cream only slowly and accordingly are probably of little practical importance in the deterioration of these products.



# STUDIES IN THE NORMAL DEPOSITIONS OF MINERALS IN THE BONES OF DAIRY CALVES\*

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The optimum development of the animal skeleton is of prime importance to the livestock man. Mineral matter comprises from 3 to 6 per cent of the animal body, about four-fifths of which is found in the skeletal frame-work. In the dairy research field much work has been done concerning the mineral requirements of mature lactating animals, but little has been reported regarding the requirements of growing animals.

Experimental studies with rats have shown that several factors including, proper amounts of calcium, phosphorus, and vitamin D, are necessary to prevent rickets and insure optimum skeletal development.

Although rickets do not appear to be common in cattle, we find considerable evidence on record that mineral deficiencies do interfere with growth and skeletal development as found in laboratory animals. A few of the outstanding references in support of this statement are as follows: Theiler and associates (13) in studying the causes of the diseases "Stijfziekte" and "Lamziekte" in South African cattle found that the addition of bone meal or other phosphorus carriers to the native rations (low in phosphorus) caused a better utilization of food and a much larger increase in body weight per unit of food consumed. Stewart (12) reports the occurrence of rickets in Australian cattle, and it appears that the disease "Stallmangel" reported by Lotsch (9) in Germany was really a form of rickets. Cases of bone disease in calves due to lack or proper utilization of minerals

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have been produced experimentally by Eckles and Swett (5), and Bechdel, Eckles, and Palmer (1).

Recently, Eckles, Becker, and Palmer (6), in reporting on rather extensive investigations on a mineral deficiency in the rations of cattle in parts of Minnesota, have shown that the skeletal development may be seriously affected by a deficiency of phosphorus in the ration. These authors, in an exhaustive search of the literature, have brought forth evidence of mineral deficiencies in the rations of cattle in many localities in the United States including, Montana, Minnesota, the coastal plain of Texas, and in the irrigated sections of the southwest and intermountain country. It is also reported in sections of the east, including eastern Michigan, and the higher altitudes of New York, Pennsylvania, West Virginia, Virginia, South Carolina, Alabama, and Mississippi, as well as many other parts of the world.

It is evident that bone diseases are not uncommon among dairy cattle. The existence of many dairy animals with an undersized skeletal development, suggests the probability of improper mineral feeding to obtain optimum body growth. Little is known concerning the amounts of calcium, phosphorus, vitamin D, or light required by the growing dairy calf. It is also entirely possible that other factors yet unknown play a part in this skeletal development. Furthermore, the most economical sources of the above factors present many problems yet unsolved.

Any satisfactory study of skeletal development requires a close examination of the structure and composition of the bones. Microscopic and x-ray examinations have proven very helpful, but the chemical composition also has been found essential for complete and thorough study. A search of the literature failed to reveal any systematic information as to the normal composition of the bones of calves at various ages. Although the main object of the present study was to make a contribution to the establishment of data on the normal composition of the bones of calves at various ages, it was also our aim to study the adaptation of technique used on such work with small animals.

## EXPERIMENTAL

Seven grade Holstein male calves were used as experimental subjects. They were purchased from farmers as bob-veals, when three to four days old. Although the individuals were not as uniform as one might desire for this type of work, the group was a fair representation of normal farm animals. The calves were kept in a box stall about 15 feet by 30 feet in size. It was equipped with individual stanchions, in which the animals were fastened at feeding time. The feeding schedule given by Eckles (7) for skimmilk calves was closely followed. The calves were started on whole milk which was gradually replaced by

TABLE 1

*Weights of calves at slaughter, normal weight, and percentage of normal calves*

ANIMAL NUMBER	AGE WHEN SLAUGHTERED	LIVE WEIGHT	NORMAL WEIGHT	PER CENT NORMAL
	<i>days</i>	<i>pounds</i>	<i>pounds</i>	
1	60	135	157	86.0
2	90	145	200	72.5
3	120	302	249	121.3
4	150	340	302	112.6
5	180	304	349	87.1
6	180	417	349	119.5
7	180	449	349	128.7

skimmilk beginning at two weeks of age. Skimmilk was fed during the entire experimental period. This was supplemented with alfalfa hay and a calf grain mixture. The calves were slaughtered at the college meat shop. One was killed at each of the following ages: 60 days, 90 days, 120 days, 150 days, while three were killed at the age of 180 days. The data on the weights of the calves at the time of slaughter are reported in table 1.

The normal weights in the above table represent the data of Eckles and associates (8) and are for Holstein females. The small size of calves 1 and 5 may be partially accounted for in that their dams were heifers, while calf 2 contracted a bad case of pneumonia while young, but had recovered at the time of slaughter.

Bones for analysis were removed from the carcasses very shortly after they had cooled. The following were selected for study: the femurs, the humeri, the fifth, sixth, and seventh pairs of ribs and a portion of the frontal bone. The humeri and femurs

TABLE 2  
*Animal 1. Age 80 days*

BONE USED	WEIGHT	VOLUME	SPECIFIC GRAVITY	GREEN BONE				ASH AS PERCENT-AGE OF FAT AND MOISTURE-FREE BONE
				Water	Fat	Ash	Organic	
	grams	cc.		per cent	per cent	per cent	per cent	
Frontal . . . . .	24	16	1.5000	33.41	2.52	39.61	26.98	61.82
Left ribs . . . . .	95	71	1.3380	47.63	2.62	29.32	23.05	58.82
Left femur . . . . .	473	390.5	1.2113	54.97	8.62	20.15	24.88	55.19
Left humerus . . . . .	314	256.5	1.2242	55.04	7.27	21.28	23.68	56.46
Right ribs . . . . .	91	67	1.3582	35.62	1.47	38.25	26.13	60.79
Right femur . . . . .	461	379.5	1.2148	32.33	6.93	39.15	28.52	64.46
Right humerus . . . . .	293	239	1.2259	35.70	7.02	36.92	27.38	64.46

TABLE 3  
*Animal 2. Age 90 days*

BONE USED	WEIGHT	VOLUME	SPECIFIC GRAVITY	GREEN BONE				ASH AS PERCENT-AGE OF FAT AND MOISTURE-FREE BONE
				Water	Fat	Ash	Organic	
	grams	cc.		per cent	per cent	per cent	per cent	
Frontal . . . . .	45	32	1.4063	41.20	2.04	32.13	26.67	56.65
Left ribs . . . . .	105	79	1.3291	44.88	2.84	29.22	25.90	55.89
Left femur . . . . .	491	407	1.2064	39.08	7.95	19.89	41.13	37.55
Left humerus . . . . .	348	286	1.2168	40.17	8.48	21.06	38.77	41.01
Right ribs . . . . .	109	84	1.2976	34.54	1.42	38.03	27.43	59.38
Right femur . . . . .	487	402	1.2114	24.18	4.47	39.26	36.36	55.02
Right humerus . . . . .	360	296	1.2162	26.47	5.18	37.41	36.12	54.73

were taken as typical examples of the long bones. These bones are also the ones commonly studied in other animals, particularly in rats. Ribs were selected because it has been shown that they are among the first to be affected in rickets. A part of the frontal was selected as an example of a membrane bone.

Immediately after removal from the carcass the bones were scraped clean of adhering tissue. They were then weighed as green bone and also weighed by suspension in water. From this data the volume and specific gravity of the bones were calculated.

TABLE 4  
*Animal 3. Age 120 days*

BONE USED	WEIGHT	VOLUME	SPECIFIC GRAVITY	GREEN BONE				ASH AS PERCENT-AGE OF FAT AND MOISTURE-FREE BONE
				Water	Fat	Ash	Organic	
	grams	cc.		per cent	per cent	per cent	per cent	
Frontal. . . . .	49	31	1.5806	35.91	1.60	37.63	26.46	60.22
Left ribs. . . . .	208	154	1.3506	44.81	3.90	29.01	26.18	56.56
Left femur. . . . .	863	699	1.2346	40.47	10.38	23.24	36.29	47.28
Left humerus. . . . .	608	491	1.2383	41.28	13.81	26.03	32.69	57.96
Right ribs. . . . .	210	155	1.3548	35.66	2.37	37.60	26.74	60.67
Right femur. . . . .	848	684	1.2398	17.32	7.57	49.97	32.71	66.53
Right humerus. . . . .	627	508	1.2343	18.59	9.00	44.65	36.66	61.75

TABLE 5  
*Animal 4. Age 150 days*

BONE USED	WEIGHT	VOLUME	SPECIFIC GRAVITY	GREEN BONE				ASH AS PERCENT-AGE OF FAT AND MOISTURE-FREE BONE
				Water	Fat	Ash	Organic	
	grams	cc.		per cent	per cent	per cent	per cent	
Frontal. . . . .	49	35	1.4000	34.88	2.69	37.25	27.87	59.66
Left ribs. . . . .	275	205	1.3415	43.85	3.57	29.50	26.65	56.10
Left femur. . . . .	935	746	1.2534	37.04	21.27	24.57	38.39	58.93
Left humerus. . . . .	686	546	1.2564	38.49	18.75	25.31	36.20	59.19
Right ribs. . . . .	275	199	1.3819	36.12	2.10	37.09	26.79	60.04
Right femur. . . . .	923	735	1.2558	22.46	12.38	44.34	33.20	68.05
Right humerus. . . . .	667	527	1.2657	23.20	10.45	44.97	32.73	67.78

In order to study the advisability of sampling, the entire bones from the left side of the animal were saved for analysis, while those from the right side were sampled by retaining about one-third from the middle of the ribs and a cross-section about an

inch long from the middle of the humerus and femur. The whole bones and portions were partially dried in a warming closet at 50°C. to facilitate grinding. The loss of moisture was then determined, and grinding was carried out immediately with a

TABLE 6  
*Animal 5. Age 180 days*

BONE USED	WEIGHT	VOLUME	SPECIFIC GRAVITY	GREEN BONE				ASH AS PERCENT-AGE OF FAT AND MOISTURE-FREE BONE
				Water	Fat	Ash	Organic	
	<i>grams</i>	<i>cc.</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
Frontal . . . . .	81	56	1.4464	36.63	2.01	35.64	27.73	58.08
Left ribs . . . . .	222	159	1.3962	39.79	3.71	33.48	26.73	59.26
Left femur . . . . .	850	679	1.2518	38.70	19.21	24.89	36.41	59.14
Left humerus . . . . .	606	480	1.2625	39.79	15.18	25.13	35.08	55.81
Right ribs . . . . .	215	152	1.4145	30.52	1.75	42.21	27.27	62.32
Right femur . . . . .	863	685	1.2599	17.69	8.56	50.84	32.37	68.94
Right humerus . . . . .	586	461	1.2711	17.18	8.79	50.36	32.46	68.03

TABLE 7  
*Animal 6. Age 180 days*

BONE USED	WEIGHT	VOLUME	SPECIFIC GRAVITY	GREEN BONE				ASH AS PERCENT-AGE OF FAT AND MOISTURE-FREE BONE
				Water	Fat	Ash	Organic	
	<i>grams</i>	<i>cc.</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
Frontal . . . . .	101	67	1.5075	33.16	1.82	38.00	28.84	58.44
Left ribs . . . . .	324	237	1.3671	40.67	3.37	32.41	26.92	57.92
Left femur . . . . .	1,240	984	1.2602	36.67	21.22	24.40	38.93	57.94
Left humerus . . . . .	900	708	1.2712	38.40	16.06	24.98	36.62	54.85
Right ribs . . . . .	313	225	1.3911	32.32	2.48	39.02	28.66	59.85
Right femur . . . . .	1,223	971	1.2595	21.75	12.07	44.35	33.90	67.01
Right humerus . . . . .	896	703	1.2745	21.34	9.90	47.01	31.65	68.37

poultry-bone grinder. Samples were weighed out in triplicate in porcelain crucibles. They were dried to constant weight in a vacuum oven at 80°C. with 15 pounds vacuum. About four hours of drying proved satisfactory for this process.

The dry samples were then quantitatively transferred to fat extraction thimbles. They were extracted for forty hours with hot alcohol by a continuous redistillation process. They were then returned to the original crucibles and dried to constant weight and the amount of fat calculated. The moisture and fat free samples were then ashed in an electric muffle furnace for twenty-four hours.

The ash was now transferred to a 250 cc. volumetric flask, dissolved in 10 cc. of concentrated HCl and made up to volume. Calcium and phosphorus determinations were made from this

TABLE 8  
*Animal 7. Age 180 days*

BONE USED	WEIGHT	VOLUME	SPECIFIC GRAVITY	GREEN BONE				ASH AS PERCENT-AGE OF FAT AND MOISTURE-FREE BONE
				Water	Fat	Ash	Organic	
	grams	cc.		per cent	per cent	per cent	per cent	
Frontal.....	58	41	1.4146	38.02	2.82	35.98	26.00	60.82
Left ribs.....	373	270	1.3815	40.00	6.44	31.44	28.56	58.70
Left femur.....	1,316	1,044	1.2605	32.00	25.93	24.75	43.25	58.83
Left humerus.....	894	699	1.2790	32.79	23.15	25.60	41.61	58.10
Right ribs.....	355	257	1.3813	29.13	5.37	39.75	31.12	60.69
Right femur.....	1,301	1,030	1.2631	21.10	17.28	42.52	36.38	69.00
Right humerus.....	885	692	1.2789	20.02	15.51	44.40	35.58	68.87

solution. The phosphorus was determined volumetrically by the official method of the Association of Official Agricultural Chemists (11). For determination of calcium the McCrudden (10) method was used.

The results of analyses are given in tables 2 to 8. Phosphorus and calcium showed such slight variation between samples that it is deemed unnecessary to report them all. The lowest percentage of phosphorus obtained was 17.90 per cent of the ash while the highest was 18.77 per cent. For calcium the extreme figures were 36.80 to 39.14 per cent of bone ash.

## DISCUSSION

The weights of the frontal bone samples are not consistent with the size of animal as only a portion of the frontal bone was removed. This portion was approximately  $1\frac{1}{2}$  by  $2\frac{1}{2}$  inches in size but varied some for the different animals. The rib weights are always the totals of the three ribs used.

The data on the weights and volumes of the bones from both the right and left halves of the body are for the entire bones. The analytical data, however, are from samples taken from the entire bones of the left side, and from portions of the bones from the right side, as stated above.

The percentages of water in green bone show that the frontal is the lowest in this constituent, followed in order by the ribs, femur, and humerus. As might be expected, the total bones contain a higher percentage of moisture than portions from the center of the bones. Another noticeable fact is the decrease in the percentage of water as the age of the animals increases.

As the fat determinations were made by calculations of loss of weight by extraction, they are not as accurate as might have been obtained by using regular ether extraction methods. But these figures will give comparative fat content and show the long bones to be the higher in this constituent.

The ash percentage shows just the reverse of the picture portrayed by the water percentages, ash being highest in the frontal followed by the ribs with the long bones lowest. As growth takes place in the ends of the bones the higher ash percentages in the central section are quite logical. The percentage of ash in bone increases somewhat with the age of the animals.

The percentages of organic matter were obtained by subtracting the sum of the ash and water percentages from 100. In general, these figures are quite uniform. A high fat content seems to indicate a high percentage of organic matter.

The last column in the tables gives the percentage of ash in the bones based on a fat and moisture free basis. These are the data most used in small animal work and seem to indicate best the condition of bone. Dutcher, Creighton, and Rothrock



(4) found that the femurs of normal rats at six weeks of age contained 62 per cent ash, while the corresponding figure for rachitic animals was 26 per cent. The percentage of ash in moisture and fat free bone probably indicates that the frontal bone is calcified at an early age. It also shows a higher degree of calcification in the center portion of the bone than in the total bone. For studies of bone development, it would, therefore, appear advisable to use the whole bone for analyses as this would include the actively growing portions at the epiphyses and would consequently show up a deficiency more clearly. It is possible that a thin longitudinal slice from the entire length of the bone

TABLE 9  
*Grams ash per cubic centimeter of bone volume*

ANIMAL NUMBER	AGE	FRONTAL	LEFT RIBS	LEFT FEMUR	LEFT HUMERUS
	<i>days</i>				
1	60	0.2641	0.2191	0.1663	0.1738
2	90	0.2285	0.2198	0.1649	0.1731
3	120	0.2381	0.2148	0.1882	0.2102
4	150	0.2661	0.2199	0.1960	0.2014
5	180	0.2464	0.2398	0.1988	0.1990
6	180	0.2686	0.2371	0.1936	0.1977
7	180	0.2456	0.2316	0.1996	0.2048

would prove as satisfactory as the whole bone. We regret that we have no data on this method of sampling.

Another interesting way to indicate the degree of calcification of bones is by the ash per cc. bone volume. These calculations could only be made where the whole bones were used as the volume of cross-section portions used for samples from the middle of the bones was not determined. These data are given in table 9.

Chick and Roscoe (2) have developed a rachitic index by using an  $A/R$  ratio.  $A$  indicates ash as per cent of fresh bone and  $R$  organic residue in fresh bone determined by subtracting the ash, fat, and water percentages from 100. This ratio is about 1:5 for normal animals and drops to 1:1 or 1:2 for rachitic animals. The above data were obtained from rats. If such a ratio were applied to the data in this work it would hold quite well for the frontal bone and the middle portions of the long

bones, but would be much lower if the entire long bones were considered. If more data become available on the composition of bones from calf skeletons such a ratio for normal animals at various ages might be worked into a convenient index for determining bone condition.

As was stated above, the percentage of calcium and phosphorus in the ash of the bones analyzed varied but slightly. Roughly, it was in the proportion of 2 to 1 for Ca and P. It is doubtful if these determinations will be of much value in the study of bone abnormalities, as the percentage of these elements in bone ash probably would not vary much even under abnormal conditions. The work of Chick, Korencheusky, and Roscoe (3), with deficient diets in rats, substantiates this view.

In the introductory statement the dearth of information on the composition of the normal bones of calves was emphasized. It is very evident that such data must be provided before constructive studies on abnormal bones can be made. Once normal data have been established, rachitic rations and other conditions that effect growth and bone development can be advantageously studied. It is appreciated that the data here presented are very meagre, but they at least represent a beginning and it is hoped that further normal data will be contributed by other investigators in this important field.

#### SUMMARY

The composition of the frontal, ribs, femurs, and humeri of seven normal male Holstein calves are presented. These calves were 60, 90, 120, 150, and three of 180 days of age. The analyses indicate that the ribs, femurs, and humeri are suitable for the study of mineral deposition in the bones of calves. Because of early calcification a section of the frontal bone is of less value. The complete bones give a truer picture of mineral deposition than a cross section portion of them. The percentage of ash based on fat and moisture-free bone and ash per cubic centimeter of bone volume proved to be the most valuable data. The ratio of ash in fresh bone to organic residue may also give a useful index of bone condition.

The percentage of phosphorus and calcium in bone ash is nearly constant and varies only slightly with the age of the animal. The percentage of water decreases with age, while the ash and organic matter percentages increase with the age of the animal. The ash replaces the greater part of the decrease in the water percentage.

Credit is due Professor R. A. Dutcher and staff of the Department of Agricultural and Biological Chemistry for advice in technique and the use of laboratory equipment in the analytical work.

#### REFERENCES

- (1) BECHDEL, S. I., ECKLES, C. H., AND PALMER, L. S. 1926 The vitamine B requirement of the calf. *Jour. Dairy Sci.*, ix, no. 5, 414.
- (2) CHICK, HARRIETTE, AND ROSCOE, MARGARET HONORA 1926 The anti-rachitic value of fresh spinach. *Biochem. Jour.*, xx, 137-152.
- (3) CHICK, HARRIETTE, KORENCHESKY, VLADIMIR, AND ROSCOE, MARGARET HONORA 1926 *Biochem. Jour.*, xx, 622-631.
- (4) DUTCHER, R. A., CREIGHTON, MATTIE, AND ROTHROCK, H. A. 1925 Vitamin studies. IX. Inorganic blood phosphorus and bone ash in rats fed on normal rachitic and irradiated rachitic diets. *Jour. Biol. Chem.*, lxi, 401-407.
- (5) ECKLES, C. H., and SWETT, W. W. 1918 Some factors influencing the growth of dairy heifers. *Res. Bulletin 31, Mo. Experiment*, pp. 3-56.
- (6) ECKLES, C. H., BECKER, R. B., AND PALMER, L. S. 1926. A mineral deficiency in the rations of cattle. *Bulletin 329, Minn. Expt. Station*.
- (7) ECKLES, C. H. 1923 *Dairy Cattle and Milk Production*. The MacMillan Company, New York., rev. ed., p. 253.
- (8) ECKLES, C. H. 1920 The normal growth of dairy cattle. *Res. Bul. No. 36, Mo. Expt. Station*.
- (9) LOTSCH, E. 1912 Über den Stallmangel, eine Eignartige Rendererkrankung in Sachsischen Erzgebirge. Zur Kenntnis des Mineralstoff Wechsels. *Z. F. Inf. U. Hyg. D. Haustiere*, 12-205.
- (10) MCCRUDDEN, FRANCES H. 1910 The quantitative separation of calcium and magnesium in the presence of phosphates. *Jour. Biol. Chem.*, vii, 83-100.
- (11) "Official and Tentative Methods of Analysis" of the Association of Official Agricultural Chemists, published by the Association of Official Agricultural Chemists, Washington, D. C., 2nd ed., 1924, p. 3.
- (12) STEWART, A. 1899 A report on the disease affecting cattle in the Moringa District. *New South Wales Agr. Jour.*
- (13) THEILER, A. 1912 Facts and theories about Stijfziekte and Lamziekte. *Agr. Jour. Union of S. Africa*.
- THEILER, A., GREEN, H. H., AND DUTOIT, P. J. 1924 Phosphorus in the live stock industry. *Agr. Jour. Union of S. Africa*, Reprint No. 18.

## THE FIRST COURSES IN DAIRY HUSBANDRY\*

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Knowledge is the great uplifting force in a civilized world. Its acquisition is, therefore, of supreme importance to human beings. Since knowledge is so all embracing, it would be comparatively useless, were it not arranged, and catalogued in an orderly manner. The organization of knowledge as well as the discovery of new facts has kept and will keep scientists busy. But in order that new generations may acquire the learning and experience of the past, teachers are needed. The teacher is one, who from the vast field of knowledge, takes such facts as will usefully develop the mind of the student and arranges and presents them in such a manner as not only to impart information but to stimulate thought. The teacher, therefore, whether of the kindergarten or the college, has not only a wonderful opportunity but a great responsibility. It is no easy task, but one requiring the greatest thought and effort.

The teacher in the college is a little likely to take teaching less seriously than is his co-worker in the secondary school. This lack of seriousness has been fostered unwittingly by the emphasis of college authorities, upon scholarship, research, and advanced degrees. There has been an all too prevalent feeling in collegiate circles that a learned man must necessarily be a good teacher. The logical deduction from this has been that there must be no suggestion that a learned man could not impart his knowledge satisfactorily to others. Unfortunately, learning does not indicate teaching ability, and the result has been that upon our faculties there have been educated fools.

An educational assumption that has frequently led to poor

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instruction, is, that the collegiate student is of mature mind. Probably this belief has descended from the ancient universities where the students were older and of more maturity. Today, however, students frequently enter college at sixteen and the average age of students is not twenty-one. In most instances also, there are many features that disturb the studious atmosphere of a college and attract and distract the student. It must therefore be borne in mind that the interest of the student must be stimulated and maintained, and that he is still a youth—as he was in high school.

In the modern world there is little time for, or little interest in, on the part of the average student, anything that does not have a practical and useful application. Today there are so many facts that at best one can master but a limited few along some few lines of endeavor. Care must be taken, therefore, not to waste the students' time with useless facts.

The first course in dairy husbandry must be carefully considered from the angles of subject matter, and the manner of presentation. It is assumed that not more than one course in dairying is required so that most students will have only this course. Consequently, the whole field of dairying will have its only presentation through this course.

#### SUBJECT MATTER

The material that is to go into the course is of vital importance and it must be weighed and considered from various angles as follows:

1. *Previous training of the student.* With the growth in numbers of Smith-Hughes Agricultural high schools, there are beginning to be taught some of the subjects that have been commonly dealt with in the first dairy course. Babcock testing, cattle judging, to give but two illustrations, are now taught in many high schools and taught well. With these subjects being covered in the secondary school, the college course must be revised, not necessarily eliminating such subjects, but modifying their presentation to avoid useless repetition.

2. *Conditions in the state.* Standardization of courses in all

states is not desirable. Each agricultural college shapes its work to train students for their own state conditions. It may be important in Wisconsin to emphasize cheese manufacture while it would be foolish to place such emphasis in Louisiana. The type of farming, and the position and condition of the dairy industry all are factors in determining what should be taught.

3. *Only one course to be given.* Too frequently the first course in dairying is designed as the logical foundation for a series of dairy courses to follow. Such an arrangement may be satisfactory if a separate course is given others, but is wrong if the course is the only one that students are required to take or are likely to take.

4. *Survey of dairying.* Assuming that the student is to get his first and only glimpse of the great dairy industry through this course, then it is essential that the course be filled with the kind of information that will correctly portray the industry, and the relation of each of the many branches. A great deal of time and effort can profitably be spent in the consideration of this one point.

5. *Time allotted.* Naturally a dairy instructor feels that the time allotted by the faculty for his course is far too short considering the importance of the subject. The time is set and good teaching demands that the maximum be crowded into the course so as to present a rounded whole. This will mean shortening here and there in order not to omit some vital part. Certain parts must not be presented in full at the expense of others, but the whole balanced to fit the time.

6. *Material of general interest.* To the specialist who has spent years of study of the subject, items of limited interest to the average person loom as important and sometimes are included in the first course. Such material should be used rarely.

7. *Divide material into general headings.* With the subject matter decided upon, then group it into related parts. For example: If dairy cattle judging is to be presented, then arrange the material that pertains to that subject together.

8. *Arrange material so that each part answers a specific problem.* In secondary school teaching this is called the problem method

and it seems well adapted to many undergraduate courses. At first it may seem the college students should be able to maintain interest over a considerable period keeping in mind the future goal. That they do not is, I believe, evident to anyone who has ever talked with agricultural students about chemistry during their first or second year. The application is too distant, too vague to interest them. The more specific the application, the greater the likelihood that it will be impressed upon the student's mind.

#### METHOD OF PRESENTATION

With the subject matter all selected, carefully arranged and and organized, there still remains the biggest problem of teaching; namely, the presentation to the class. A class, remember, that is composed of immature minds affected with a thousand distractions and compelled by our present system of class hours to attempt to absorb the reason for the reactions of certain chemicals one hour, the principles of English composition the following period, and be interested in and show enthusiasm for the life history of the algae the next period. Add to this, the fact that frequently the classes range from thirty to forty so that little individual attention may be given, and it is not surprising that all too frequently, the first course in dairying has become merely a series of lectures, with a text book assignment from time to time and a quiz to determine the class grade. As opposed to this method, our plan is to try to make the course so practical and interesting, that the student will appreciate the value and will study because he believes it is worth while. The preparation of the material to be given to the class and the actual presentation are so closely interwoven that they will be discussed together.

1. *Give proper emphasis.* Giving the proper emphasis to the essential facts saves the student time and creates a better spirit within the class. The average student greatly dislikes to have his time wasted by being compelled to remember a lot of unimportant facts.

2. *Make each period interesting.* It may seem puerile to some, that a college class presentation needs to be interesting but it is

exceedingly important. Frequently, the form of presentation that is most appealing to students may seem illogical to one with a greater knowledge yet if it will gain and keep attention it is worthy of use.

3. *Make the class work provoke thought and reasoning.* It has been said that if a college course stimulates thought, it is successful even if it conveys no information. There is sometimes a tendency for the instructor to appear in class and give a lecture which is merely a recitation of known facts all of which may be found better arranged in a book. Such a lecture may stimulate thought, but it is unlikely.

4. *Make the object clear.* The aim or underlying thought that each class period is to bring out or the part of the whole that is to be emphasized, should be stressed. Always keep the immediate object of the problem before the class and at the same time show its relation to the larger problem covered by the course.

5. *Each part should be a step toward the whole.* Each lesson should fit in toward the main goal and the information obtained should better fit the student to solve the next problem before him.

6. *Make the student work.* Each student is required to have the course outline. There the object or problem is outlined for each class period and for each laboratory period. In the outline for a class period the questions are asked that are to be answered or discussed at that period. References are given also where information may be found upon the subject. In the class period, the instructor brings out by class discussion the various opinions and ideas of the students, corrects misapprehension and shows the relationship of the problem to the whole subject. Under such a plan the student must work, since it is his reaction to the problem that is brought out and not a mere recitation of known facts.

With these principles in mind, at Nebraska we have tried to build a course in first year dairying that would be suitable for our State, for a three-hour course (two one-hour class periods and two two-hour laboratory periods), for men that probably had no dairy background, and may never have another dairy course.



## OUTLINE OF CLASS PERIODS AND LABORATORIES

In arranging the class periods and laboratory exercises, it has been necessary to consider the relationship of one to the other as well as the logical development and the retention of interest. The arrangement given seems to give the most satisfaction under our conditions. Under our conditions, the laboratory period always follows the class period.

<i>Class Period</i> <i>Subject</i>	<i>Laboratory Period</i> <i>Subject</i>
1. Relation of dairying to agriculture; how it fits into agricultural practice. Future for the dairy industry.	1. Observation trip through college dairy barn and creamery. Attention is called to methods used in handling dairy cattle and dairy products. Mimeograph sheets of department's activities are given to each student to show the scope of dairying as existing at the college.
2. Relation of form to function in dairy cattle. What is a dairy cow? How different from other cows?	2. Use of score card in judging dairy cows. Application of the principles discussed in the class period.
3. Origin of cattle types. A background for studying breed characteristics.	3. Judging Ayrshire cows—comparative judging using only the essential parts of the cow.
4. Ayrshires—history, characteristics and present usefulness.	4. Judging Ayrshire—continuation of comparative judging.
5. Holstein-Friesians—history, characteristics and present usefulness.	5. Judging Holsteins—comparative. Same as first period with Ayrshires.
6. Guernseys—history, characteristics and present usefulness.	6. Judging Holsteins—comparative. Same as second with Ayrshires.
7. Jerseys—history, characteristics and present usefulness.	7. Judging Guernseys—comparative.
8. Brown Swiss, minor and dual purpose breeds, characteristics and present usefulness.	8. Judging Guernseys—comparative.
9. Methods of starting a dairy herd. The advantages and disadvantages of the different breeds having been discussed, the actual considerations necessary in selecting and buying dairy cows are brought out.	9. Judging Jersey's—comparative.

10. The herd sire, important characteristics, method of selection, care and feed.
11. Feeding—general principles.
12. Feeding dairy cows in summer.
13. Feeding dairy cows in winter.
14. Care of the cow, before, at and immediately after freshening.
15. Feeding and care of the dairy calf.
16. Midsemester examinations.
17. Sampling of milk, plain and composite; the use of preservatives. Explanation of the operation of the Babcock test for milk.
18. Principles of the Babcock test; causes and remedies; defective tests.
19. Babcock test for cream, skim-milk, buttermilk and whey.
20. Milk secretion.
21. Milk composition.
22. Standardization of dairy products.
23. Cream separation.
24. Choosing and operating a separator.
25. Bacteria and their relation to milk.
26. Care of milk and cream on the farm, milking machines.
27. Principles of buttermaking.
28. Principles of cheese making.
29. Principles of ice cream making.
30. Food value of dairy products.
31. Methods of selling milk.
32. Visit to a dairy farm, milk plant and dairy manufacturing plant.
33. Final examination.
10. Judging Jersey's—comparative.
11. Judging dairy bulls for type.
12. Selection of dairy cows and bulls as an investment.
13. Selection of dairy cattle as an investment. Continuation of 12.
14. Balancing rations to fit common roughages.
15. Barn practice—dehorning calves, ringing bulls, clipping, trimming hoofs, etc.
16. Midsemester examinations.
17. Testing whole milk by the Babcock test.
18. Testing whole milk by the Babcock test.
19. Testing cream by the Babcock test.
20. Testing cream by the Babcock test.
21. Keeping cow testing association records.
22. Practice in standardization.
23. Testing skim-milk, buttermilk and whey.
24. Study of the mechanical construction of a separator.
25. Operation of cream separators.
26. Cooling and care of milk.
27. Demonstration of churning butter commercially in the creamery.
28. Demonstration of making cheese in the creamery.
29. Demonstration of ice cream making in the creamery.
30. Judging butter, cheese and milk.
31. Calculation of returns from different methods of selling milk.
32. Visit to dairy plants.
33. Final examination.

The lectures and laboratories are not arranged in the exact order that is desired, but they are arranged so that as far as possible, the laboratory will follow the lecture period where the subject is discussed. There may be some difference of opinion as to the desirability of including some subjects under a first year course. From our standpoint and under our conditions this arrangement is believed to be most satisfactory.

#### CLASS PERIODS METHODS AND MATERIAL

In the first class period usually there has been no chance for preparation so that with the outlines before the class the instructor proceeds to answer the questions bringing out discussion wherever possible.

#### LESSON 1

##### RELATION OF DAIRYING TO AGRICULTURE

*Object.* To present a brief survey of the dairy industry and to indicate the value of dairy training.

1. What is dairying?
2. Why is the subject of dairying included in an agricultural course?
3. What are the usual steps or stages in agricultural development? Explain the reason for this sequence of development.
4. What are the advantages and disadvantages of dairying as a business?
5. What are the different types of dairy farms? What are the local conditions that make for the success of each type?
6. What will be the use of dairy training to you as an average student?
7. In order to show the extent of the dairy industry in the United States and Nebraska tabulate for the next period the following:
  - a. Number of dairy cattle in U. S., Nebraska and home county? How many dairy cows per square mile in each?
  - b. Pure bred dairy cattle in U. S. and in Nebraska?
  - c. Value of dairy cattle in U. S., in Nebraska and home county? Compare with total live stock values in each.
  - d. Value of dairy products produced in U. S., in Nebraska, and home county? Compare with other agricultural products.
  - e. Total milk production in U. S., in Nebraska, and home county?
  - f. Uses of milk in U. S. by percentages?
  - g. Compare per capita consumption of milk, butter and cheese in the U. S., Switzerland, Denmark and Germany?

At the first class period, a questionnaire is given each student asking the following information. This is an endeavor to get the

background of the student in order to present the course most effectively.

### GENERAL INFORMATION FROM STUDENTS

Give the following information in regard to your own experiences:

1. Do you live on a farm? If the answer is "Yes" answer all questions; if "no" answer only those questions that are starred\*.
2. Do you milk cows on your home farm? How many?
3. Is dairying a minor or major part of the business on your farm?
4. What breed of cows are used on your farm?
5. Are the cows grades or pure breds?
6. What breed of cows prevail in your community?
7. Is a pure bred bull used on your farm?
8. Do you know the yearly production of each of your cows?
9. What means of keeping milk records is used?
10. What is the average production per cow per year in your herd?
11. Do you have a milking machine? What kind? Is it satisfactory?
12. In what form do you sell your milk? Why?
13. Do you have a cream separator? What kind? Why?
14. What part of the total feed for your cows do you raise on the farm?
- 15.\* Do you consider dairying a profitable business?
16. What are the main crops on your home farm?
- 17.\* What do you expect to do when through college? Why?
- 18.\* What are some of the main points that you would like to get from this first course in dairying?
- 19.\* Do you expect to take other dairy courses?
- 20.\* What has been the extent of your dairy training before coming to college?
- 21.\* How many dairy plants of any kind are there in your town? What do they manufacture?
- 22.\* Have you ever visited a large dairy plant and observed the handling of dairy products on a large scale?

From this information it is hoped to tie the students' local interest to the work presented in the course. The questionnaire used is designed to be used with a class that contains a large majority of farm-raised boys.

It should be mentioned, that at the first class period use of the outline and the reference books is explained. The next lesson or problem is outlined also and any needed explanation made.

### LESSON 2

#### RELATION OF FORM TO FUNCTION IN DAIRY CATTLE

*Object.* To determine the importance of judging in the selection of dairy cows.

1. How would you select a dairy cow for your own herd?

2. What is meant by dairy type? Compare dairy with breed type and explain differences?
3. What is a score card? What is it's purpose?
4. What points are most emphasized on the score card and why?
5. How does a breed score card differ from general dairy cattle score cards? Why?
6. What is meant by the following terms: breed, pure bred, scrub, grade, pedigree, family?
7. What are the purposes of breed associations?

The student should appear at the second class period prepared to answer these questions all of which are discussed in the list of references given in the outline. The instructor then must bring out by class discussion the reaction of the student to the problem of the relation of form to function. The instructor should also correct any false impression that may have developed.

### LESSON 3

#### ORIGIN OF DAIRY CATTLE TYPES

*Object.* To bring out by the study of the origin of cattle, the present day types and the breed characteristics.

1. For what purposes have cattle been developed?
2. What are the milk producing animals of the world? Why are different animals used in different countries?
3. Compare present day dairy cow with the early cows?
4. What has brought about the change in dairy cows?
5. What are ruminants? For what purposes are they best fitted? Name several common ruminants?
6. What are the chief characteristics of the division of the genus *Bos*; *Bos Indicus*; *Bos primigenius* and *Bos Longifrons*? What breeds today show these characteristics?
7. Describe and explain two ways in which types of cattle may develop?

From that lesson a ground work is laid upon which the breeds of dairy cattle may be discussed.

### LESSON 4

#### DAIRY BREEDS—AYRSHIRE

*Object.* To bring out by a discussion of history and characteristics the usefulness of each particular breed.

1. (a) Name the major breeds of dairy cattle?  
(b) Name the minor and dual purpose breeds?
2. Why have some breeds developed to a great extent than others? Explain answer.

3. Considering the original environment, the character of the people, etc., is the Ayrshire cow the type that you would expect? Why?
4. What in detail are the characteristics of the Ayrshire?
5. For what environmental and market conditions would you select Ayrshires? Explain.
6. Where are the largest numbers of Ayrshires in the United States? Why?
7. What are the number of pure bred and grade Ayrshires in United States and in Nebraska? How does the breed compare in importance in United States and Nebraska?
8. What is the average production of Ayrshire advanced registry cows in milk and fat? What is the average per cent of fat?
9. What particular characteristics has Ayrshire milk? For what purpose is it especially useful?
10. Name five of the leading milk producing cows of the Ayrshire breeds and give production?
11. Name and locate the organization that registers Ayrshire cattle in the United States?

In this lesson an effort is made to consider cattle that are used for dairy purposes and then to bring out in some detail one breed, the Ayrshire. In the class discussion led by the instructor local relationships can be established.

If it were to be summarized in outline form, our conception of the first course in dairy husbandry would be somewhat as follows:

- I. Planning the course.
  1. According to training of the student.
  2. With reference to conditions in the state.
  3. As the only course that students will take.
  4. To present a survey of the dairy industry.
  5. To meet the allotted time.
  6. To cover material of general interest.
  7. Arrangement of subject matter under general headings.
  8. Each part to answer a specific question.
- II. Presentation of the course.
  1. Proper emphasis.
  2. Interesting.
  3. Provocative of thought and reasoning.
  4. Object clearly set out.
  5. Each part a step toward the whole.
  6. Make the students work.

# BACTERIAL ACTION IN THE COAGULATION OF EVAPORATED MILK\*

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The coagulation of unsweetened condensed (evaporated) milk has occasioned considerable loss to the condensed milk undustry. Instances have been recorded of the spoilage of large numbers of cans, sometimes involving practically entire batches and, again, occurring frequently enough among the cans of single batches to render exceedingly doubtful the delivery of the milk into the hands of the consumer in a perfect, sweet, sterile condition. Chemical composition has been important in limiting the extent of sterilizing and excessive percentages of solids have in some cases been responsible for coagulation upon sterilizing. In others the high percentage of solids has induced incomplete sterilization because of the effort to prevent curdling during the exposure to heat.

Curdling due to bacterial action after sterilization has been recognized and Hammer (1) has isolated and described an organism which he encountered as a cause of coagulation and spoilage involving considerable loss to the condensery experiencing the trouble. He named this organism *Bacillus coagulans*.

## PRESENT INSTANCE OF TROUBLE

The work herein reported was done in studying an outbreak of trouble in a condensery due to the coagulation of evaporated milk after sterilization and development of so-called "flat-sours." It had occasioned considerable anxiety on account of the sporadic nature of the epidemic and the failure of a number of changes in methods of manufacture to stop the trouble. From the information given it was impossible to judge whether the difficulty was a chemical one, involving a limitation in the percentage of total solids permitting sterilization by heat, or a bacteriological dis-

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turbance caused by the action of bacteria surviving the heat exposure during sterilization.

#### EXAMINATION OF MILK SUBMITTED

Several cans of the curdled evaporated milk were submitted. After removal of samples for bacteriological study an examination was made of the contents. The milk was found to be definitely curdled with irregular masses and lumps of curd present, and to possess a slightly bitter flavor of a cheesy nature but not unpleasant. No free whey was apparent and no gas-escape was noted upon opening. None of the cans submitted was bulged.

While at the outset there was no information available indicating whether the defect was of bacteriological nature or a result of an unfortunate high solids composition, the physical examination of the curdled milk indicated biological activity. This was confirmed by the plating of the milk for determination of viable bacteria. One can gave a count of 9,000,000 colonies per cubic centimeter and another showed but 75,000. Several weeks elapsed between platings and it is likely that considerable numbers died out in the interval. Direct transfers were made at time of plating from the milk in the cans to tubes of litmus milk, which were incubated to allow enrichment of any organisms present. Such tubes curdled after a number of days, depending on the temperature used, and were also plated for study of the organisms present and isolation in pure culture.

The same organism was obtained from all sources examined—plating of original cans and of enrichment tubes—and the milk in the cans appeared to be essentially a pure culture. The organism isolated was found, upon study morphologically, culturally, and biochemically, to correspond with the *Bacillus coagulans* of Hammer.

#### STUDY OF ORGANISM ISOLATED

Observations recorded here constitute merely a brief survey of a few of the important characters of the organism isolated, serving to mark its identity as *B. coagulans*.

Morphologically, the causative organism was rod-shaped, gen-



erally occurring singly, Gram-positive in young cultures, but predominantly negative in old. The predominating size in young agar cultures was 3.5 to 5.0 microns long and 0.5 micron wide. Occasional cells had the appearance of containing spores but these were not at all distinct. The water of condensation in twenty-four-hour whey agar slope cultures showed the rods to be actively motile.

The growth on a whey agar slant was fair in amount after twenty-four hours at 37°C., echinulate, glistening, whitish, slightly raised, and increasing in amount with age, with heavy turbidity in the water of condensation. Development was much slower at room temperature.

The colony on whey agar was 1 to 2 mm. in diameter after four days at 37°C., circular with entire edge, slightly raised, glistening, and whitish; clouded areas occurred surrounding the colonies, due to the penetration of acid into the medium. At room temperature colonies were pin-point in size after one week.

In litmus milk a slight reduction was evident in the bottom of the tube after twenty-four hours at 37°C. Complete coagulation occurred usually after three days with young, vigorous cultures; a red band formed at the surface, and there was a slight contraction of the curd in some cases; no gas was formed. At room temperature about five days was required for the first change to appear, and ordinarily coagulation occurred in about thirty days.

The acidity production was studied in flasks of sterilized skim milk inoculated with the organism and held at different temperatures. Beginning coagulation was noted at acidities as low as 0.38 per cent and 0.39 per cent, thus confirming the results secured by Hammer in the original isolation of *B. coagulans* and indicating the activity of a rennin-like principle in the production of initial coagulation. Total acid production at 37°C. after fifteen days ranged around 0.85 to 0.90 per cent.

#### REPRODUCTION OF DEFECT BY ARTIFICIAL INOCULATION

In order to demonstrate conclusively the relationship of the organism isolated to the abnormal, curdled condition of the original milk submitted, inoculation experiments were carried out

on cans of normal evaporated milk. The usual method of introducing suspensions of the organism through a hole punched in the tin with a sterile nail was followed. Control cans were opened and in some cases sterile water added before soldering over. The cans were incubated at both 37°C. and room temperature. They commonly exhibited a slight bulging after several days at 37°C. The curdled, lumpy condition of the original spoiled milk was reproduced, accompanied by the slightly bitter, cheesy flavor of the cans submitted. The odor was characteristically sweetish, slightly cheesy. This condition was obtained in cans held ten days at 37°C. Inoculated cans allowed to stand without agitation were found to have the contents in a solid cake, more or less firm according to the temperature and time of incubation. Acidity production was around 0.9 to 1.0 per cent. The inoculated cans were examined bacteriologically and found to be pure cultures of *B. coagulans*, the organism inoculated.

#### THERMAL DEATH POINT OF ORGANISM

Having demonstrated that the coagulation in the original cans was due to bacterial action, it became necessary to determine how resistant the organism was to heat. The cans submitted had been sterilized in a batch exposed to 238°F. for twenty minutes and were typical of a number of cases of so-called "flat-sour" that developed some time after sterilization.

Preliminary tests with various cultures showed that the organism survived the ordinary test for spores, 80°C for ten minutes. Milk was the medium used and fresh transfers were used for heating, the milk itself being kept at a temperature of 80°C. for ten minutes.

Further trials with fresh milk transfers of several cultures were made by heating the tubes of litmus milk in a bath of boiling water. The data in table 1 are typical of the temperature changes found, the tubes of cultures and the water bath both being at 85°F. to start, and an extra tube of milk being placed with the culture tubes for the thermometer.

Tubes were cooled down quickly in cold water after taking out of the bath. These results showed that the organism could

withstand three minutes exposure to temperatures practically that of boiling water, where the temperature of the culture itself reached 210°F. The milk cultures showed the usual changes—reduction of the litmus and coagulation—after several days incubation at 37°C.

Further trials for thermal death point were made with the autoclave, using steam under pressure, fresh milk cultures in cotton-stoppered test tubes being used as before. These were placed in a container together with a tube of milk in which a maximum registering thermometer was placed. The container was placed in the center of the autoclave and a second registering

TABLE 1

TIME	TEMPERATURE		TUBE NUMBER	GROWTH ON INCUBATION
	Bath	Tube		
3:10 p.m. (start)				
3:11	100			
3:11½		100		
3:13	140	126		
3:14½	170			
3:15	180	164		
3:16		176		
3:17	208	192	1	+
3:18	Boiling	207	2	+
3:19		210	3	+
3:20		210	4	+

thermometer placed on a stand beside it, the autoclave being otherwise empty. In this way temperatures were obtained of both the autoclave and the cultures themselves. Cultures from different isolations were used; after autoclaving, the pressure was let down as rapidly as possible and the tubes cooled in water; incubation was at 37°C.

Under these conditions cultures survived where the milk medium actually reached 223°F., the temperature being 223° for two minutes, between 212° and 223° for five minutes, and between 193° and 223° for twelve minutes. The temperature of the autoclave to which the milk was exposed was 233° to 234°

for thirteen minutes (fifteen minutes at about 7.5 pounds pressure). In other trials cultures reached 225° and survived, the time of exposure to the maximum temperature in the autoclave being twelve minutes, and the milk tubes being 212° to 225° for four minutes.

Cultures exposed to a temperature in the autoclave of 233° to 235° for fifteen minutes (seventeen minutes at 7.5 to 8.0 pounds pressure) were killed, the milk itself reaching 234° and being between 212° and 234° for six minutes, and between 225° and 234° for four minutes.

#### REFERENCE

- (1) HAMMER, B. W.: Bacteriological studies on the coagulation of evaporated milk. Iowa Agr. Exp. Sta. Res. Bull. 19, 1915.

# VARIABILITY IN COMPOSITION OF BUTTER FROM THE SAME CHURNING IN RELATION TO WORKING\*

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Uniformity in the distribution of the water, salt, curd, and milk fat in creamery butter is highly desirable but seldom attained to the satisfaction of exacting creamerymen. This lack of uniformity is evidenced by the variation in the composition of butter prints from the same churning. It is noticeable in the product of the best managed creameries, where careful attention is given to composition control, and often results in unintentional violation of governmental regulations pertaining to the composition of butter. This station found that over 30 per cent of the prints sold at retail markets contain less than 80 per cent milk fat, while over 20 per cent of the prints contain 16 per cent or more water. Most creamerymen believe that such violations may be largely attributed to the nonhomogeneous character of butter.

According to Lee, Hepburn, and Barnhart (1) there is a variation in water content, ranging from 0.1 to 1 per cent, between different samples representing butter from the same churning. Hunziker, Mills, and Spitzer (2) found the following averages for water content in butter from different parts of the churn: gear end, 15.91 per cent; middle, 15.44 per cent; and gate end, 16.33 per cent. Guthrie and Ross (3) found 17.6 per cent of 51 packages with a difference of 1 per cent or more water in adjacent samples, 54.9 per cent with a difference of 0.5 per cent; while in 34.2 per cent of the packages, there was a difference of 0.2 per cent salt in adjacent samples.

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The results of these investigations show a large variation in the distribution of water and salt in butter. It is generally understood that insufficient working of butter may cause excessive variation in composition, and some butter experts advocate that the working process be prolonged until the texture of the butter is very close and the body quite dry. Such working, it is said, will result in less variation in the composition of butter coming from the same churning.

The investigation reported in this paper was conducted for the purpose of determining the effect of prolonged working of butter upon the distribution of the water, salt, curd, and fat content.

#### MATERIALS AND METHODS

Butter used in the investigation was manufactured at the Purdue Creamery in a single roll Cherry Dreadnaught Churn, No. 23 with a capacity (half full) of 3033 pounds of cream. The size of the churnings were varied from 876 to 2257 pounds of cream. All churnings were made during the months of December, 1926, January and February, 1927.

The samples consisted of 300 grams of butter taken from the churn with a trier and transferred to pint size, glass stoppered jars. The preparation of the samples, and the water and fat analyses were done in accordance with the methods of analysis of the Association of Official Agricultural Chemists (second edition, paragraphs 67, 68 and 69). The salt and curd contents were determined as follows. The salt was washed from the fat free solids, remaining in the crucible after the extraction of the fat, and titrated with silver nitrate. The difference between the fat free solids and the salt content was taken as the curd content.

#### EXPERIMENTAL

Ten churnings were made from which samples of butter were obtained for analysis. Nine samples were taken from each of the ten churnings at the following stages of the working process: three samples when the butter had been worked thirty revolutions, three when worked forty-five revolutions, and three when

worked sixty revolutions. Of each set of three samples thus taken, one sample was taken at the gate end of the churn, one from the middle, and a third at the gear end of the churn. The samples were obtained by inserting an eighteen-inch butter trier the full depth of the butter roll in the churn and transferring the entire plug to the sample jar. The operation was repeated until the full sample was obtained, care being taken to avoid surface water pockets and drippings from the wall of the churn.

The cream used in the churnings consisted of the regular run of farm skimmed cream received at the Purdue Creamery. It was standardized to 0.25 per cent acid with magnesia lime and then flash pasteurized at a temperature of 185°F., cooled over a

TABLE 1

*Churning data pertaining to the cream used in the ten churnings of the experiment*

CHURNING NUMBER	AMOUNT OF CREAM	FAT CONTENT	RIPENING PERIOD	RIPENING TEMPERATURE	CHURNING TEMPERATURE
	<i>pounds</i>	<i>per cent</i>	<i>hours</i>	<i>°F.</i>	<i>°F.</i>
1	876	30.5	3	50	50
2	1,046	32.0	4	52	53
3	1,175	34.0	3	48	48
4	1,352	33.5	1	52	52
5	1,288	35.5	3	50	50
6	1,483	33.0	2	52	53
7	1,661	32.0	3	52	52
8	1,669	35.0	1	50	50
9	1,969	35.0	1	53	53
10	2,257	33.5	1	50	50

tubular surface cooler to near the churning temperature and ripened in a vat ripener, there being about 10 per cent starter added. The pounds of cream, its milk fat content, ripening period and temperature, and the churning temperature for each churning are shown in table 1.

The cream was churned until the butter granules became a trifle larger than grains of wheat. The temperature of the wash water was 4°F. less than that of the buttermilk. The water was drained from the churn through the gate, and then the butter was worked five revolutions. The doors were then opened a

TABLE 2

*Variation in the water content of butter from different parts of the churn as affected by working*

NUMBER OF REVOLUTIONS WORKED	CHURNING NUMBER	PERCENTAGE OF WATER					
		Gate end	Middle	Gear end	Average of the three	Average deviation	Coefficient of variability
30	1	14.73	14.85	14.52	14.70	0.120	0.82
	2	13.62	13.43	13.29	13.45	0.118	0.88
	3	14.11	14.08	13.93	14.04	0.073	0.52
	4	13.62	13.99	13.60	13.74	0.171	1.24
	5	13.94	13.88	13.99	13.94	0.038	0.27
	6	14.11	14.34	14.20	14.22	0.084	0.59
	7	14.73	15.03	14.78	14.85	0.124	0.83
	8	15.55	15.67	15.33	15.52	0.128	0.82
	9	14.52	14.77	14.45	14.58	0.127	0.87
	10	14.10	14.13	13.96	14.06	0.069	0.49
Average.....		14.30	14.42	14.20	14.30	0.105	0.73
45	1	15.11	14.82	14.94	14.96	0.104	0.70
	2	14.07	13.90	13.88	13.95	0.080	0.57
	3	14.92	14.66	14.41	14.66	0.169	1.15
	4	14.17	14.09	14.19	14.15	0.040	0.28
	5	14.97	15.00	14.98	14.98	0.009	0.06
	6	15.00	15.55	15.35	15.30	0.200	1.31
	7	15.60	15.61	15.50	15.57	0.047	0.30
	8	16.72	17.05	16.62	16.79	0.171	1.01
	9	15.19	14.91	14.70	14.93	0.169	1.13
	10	14.07	14.03	13.75	13.95	0.133	0.95
Average.....		14.98	14.96	14.83	14.93	0.112	0.75
60	1	14.81	14.88	15.08	14.92	0.102	0.68
	2	14.26	14.11	13.86	14.08	0.144	1.02
	3	15.48	14.98	15.13	15.20	0.191	1.26
	4	15.00	15.06	14.94	15.00	0.040	0.27
	5	15.96	15.97	15.98	15.97	0.007	0.04
	6	15.93	16.45	16.20	16.19	0.176	1.09
	7	16.03	16.55	16.46	16.35	0.211	1.29
	8	16.92	17.26	16.94	17.04	0.147	0.86
	9	15.15	15.02	14.75	14.97	0.149	0.99
	10	14.29	14.21	13.99	14.16	0.116	0.82
Average.....		15.38	15.45	15.34	15.39	0.128	0.83



trifle and the churn rotated so as to permit the remnants of wash water to drain through them. The butter was salted by the trench method at the rate of 4 pounds of salt to 100 pounds of fat, after which, the churn was closed tight and the working continued without further attempt to regulate the composition of the butter.

### *Results*

The effect of prolonged working upon the distribution of the water, salt, curd, and fat in butter is shown by tables 2, 3, 4, and 5, respectively. These tables, in addition to the original data, show the average deviations and the coefficients of variability of the three samples taken from each churning at the different stages of the working process, namely; thirty, forty-five, and sixty revolutions.

In table 2 it will be noted that the averages of the coefficients of variability indicate that the distribution of water become more variable as the working process was prolonged. However, this is not true of individual churnings. Churning 1, 4, and 5 show the opposite effect, while 2 and 7 show a less variable distribution of water at forty-five revolutions than at thirty, but a more variable distribution at sixty revolutions. Churning number 3 alone shows an increase in variability throughout the working process; while in churnings 6, 8, 9, and 10 the variability decreased from forty-five to sixty revolutions, yet it remained greater at sixty than at thirty. In these churnings, size of the churning undoubtedly exerted some influence upon the distribution of water. The size of the churnings increased in the order of their numbers, from number 1, the smallest, to number 10, the largest, as may be recalled from table 1. Table 2 shows that in all of the five larger churnings, numbers 6 to 10 inclusive, the variability is greater at sixty revolutions than at thirty, likewise, in four churnings, greater at forty-five revolutions than at thirty; while but two churnings of the five smaller ones show greater variability at sixty revolutions than at thirty, and only one churning shows a greater variability at forty-five revolutions than at

TABLE 3

*Variation in the salt content of butter from different parts of the churn as affected by working*

NUMBER OF REVOLUTIONS WORKED	CHURNING NUMBER	PERCENTAGE OF SALT					
		Gate end	Middle	Gear end	Average of the three	Average deviation	Coefficient of variability
30	1	2.73	2.74	2.23	2.57	0.224	8.73
	2	3.27	3.49	4.00	3.59	0.278	7.75
	3	2.78	2.65	2.54	2.66	0.084	3.16
	4	2.32	2.27	1.80	2.13	0.220	10.33
	5	2.60	2.60	2.40	2.53	0.089	3.51
	6	2.78	2.40	1.77	2.32	0.364	15.71
	7	2.29	2.41	2.25	2.32	0.064	2.76
	8	2.18	2.34	2.58	2.37	0.144	6.08
	9	3.40	3.56	2.84	3.27	0.284	8.69
	10	3.19	3.66	3.73	3.53	0.224	6.35
Average.....		2.75	2.81	2.61	2.73	0.198	7.31
45	1	2.87	2.58	2.39	2.61	0.169	6.47
	2	3.53	3.84	4.09	3.82	0.193	5.05
	3	2.99	2.75	2.62	2.79	0.102	3.66
	4	2.49	2.34	2.14	2.32	0.122	5.25
	5	2.69	2.81	2.88	2.79	0.069	2.47
	6	3.00	2.79	2.23	2.67	0.296	11.07
	7	2.60	2.69	2.50	2.60	0.064	2.46
	8	2.39	2.77	2.87	2.68	0.191	7.13
	9	3.63	3.61	3.00	3.41	0.276	8.09
	10	3.24	3.50	3.66	3.47	0.151	4.36
Average.....		2.94	2.97	2.84	2.92	0.167	5.73
60	1	2.84	2.74	2.56	2.71	0.102	3.76
	2	3.64	3.78	4.02	3.81	0.136	3.57
	3	3.09	2.90	2.93	2.97	0.076	2.56
	4	2.74	2.62	2.40	2.59	0.124	4.79
	5	3.07	3.08	3.15	3.10	0.033	1.06
	6	3.18	3.16	2.57	2.97	0.267	8.99
	7	2.75	2.91	2.84	2.83	0.056	1.98
	8	2.57	2.81	3.01	2.80	0.151	5.41
	9	3.63	3.64	3.06	3.44	0.256	7.43
	10	3.21	3.56	3.73	3.50	0.193	5.51
Average.....		3.07	3.12	3.03	3.07	0.139	4.51

TABLE 4  
*Variation in the curd content of butter from different parts of the churn as  
 affected by working*

NUMBER OF REVOLUTIONS WORKED	CHURNING NUMBER	PERCENTAGE OF CURD					
		Gate end	Middle	Gear end	Average of the three	Average deviation	Coefficient of variability
30	1	0.69	0.61	0.61	0.64	0.038	5.96
	2	0.65	0.74	0.72	0.70	0.036	5.12
	3	0.76	1.03	1.26	1.02	0.171	16.81
	4	0.79	0.79	0.86	0.81	0.029	3.56
	5	0.52	0.55	0.62	0.56	0.031	6.39
	6	0.83	0.72	0.73	0.76	0.047	6.18
	7	0.81	0.85	0.89	0.85	0.027	3.18
	8	0.52	0.56	0.63	0.57	0.040	7.02
	9	0.70	0.62	0.61	0.64	0.036	5.60
	10	0.58	0.57	0.43	0.53	0.064	12.14
Average.....		0.68	0.70	0.74	0.71	0.052	7.20
45	1	0.60	0.74	0.74	0.69	0.062	8.94
	2	0.59	0.57	0.66	0.61	0.038	6.26
	3	0.96	0.75	1.16	0.96	0.138	14.42
	4	0.60	0.64	0.64	0.63	0.018	2.87
	5	0.53	0.48	0.54	0.52	0.024	4.64
	6	0.77	0.86	0.81	0.81	0.029	3.57
	7	0.66	0.57	0.77	0.67	0.071	10.64
	8	0.61	0.73	0.69	0.68	0.044	6.49
	9	0.69	0.69	0.71	0.70	0.011	1.58
	10	0.55	0.52	0.61	0.56	0.033	5.89
Average.....		0.66	0.66	0.73	0.68	0.047	6.53
60	1	0.70	0.73	0.77	0.73	0.022	3.00
	2	0.63	0.57	0.72	0.64	0.053	8.28
	3	0.89	1.00	1.01	0.97	0.051	5.27
	4	0.57	0.87	0.80	0.75	0.118	15.79
	5	0.73	0.75	0.75	0.74	0.009	1.21
	6	0.83	0.77	0.78	0.79	0.022	2.77
	7	0.81	0.82	0.82	0.82	0.003	0.37
	8	0.78	0.71	0.69	0.73	0.038	5.23
	9	0.54	0.62	0.65	0.60	0.042	6.96
	10	0.54	0.51	0.60	0.55	0.033	6.00
Average.....		0.70	0.74	0.76	0.73	0.039	5.49

TABLE 5

*Variation in the fat content of butter taken from different parts of the churn as affected by working*

NUMBER OF REVOLUTIONS WORKED	CHURNING NUMBER	PERCENTAGE OF FAT					
		Gate end	Middle	Gear end	Average of the three	Average deviation	Coefficient of variability
30	1	81.85	81.80	82.64	82.10	0.364	0.44
	2	82.46	82.34	81.99	82.26	0.182	0.22
	3	82.35	82.24	82.27	82.29	0.044	0.05
	4	83.27	82.95	83.74	83.32	0.280	0.34
	5	82.94	82.97	82.99	82.97	0.018	0.02
	6	82.28	82.54	83.30	82.71	0.398	0.48
	7	82.17	81.71	82.08	81.99	0.184	0.22
	8	81.75	81.43	81.46	81.55	0.138	0.17
	9	81.38	81.05	82.10	81.51	0.393	0.48
	10	82.13	81.64	81.88	81.88	0.162	0.20
Average.....		82.26	82.07	82.44	82.26	0.216	0.26
45	1	81.42	81.86	81.93	81.74	0.178	0.22
	2	81.81	81.69	81.37	81.62	0.169	0.21
	3	81.13	81.84	81.81	81.59	0.309	0.38
	4	82.74	82.93	83.03	82.90	0.107	0.13
	5	81.81	81.71	81.60	81.71	0.071	0.09
	6	81.23	81.80	81.61	81.55	0.211	0.26
	7	81.14	81.13	81.23	81.17	0.044	0.05
	8	80.28	79.45	79.82	79.85	0.287	0.36
	9	80.49	80.79	81.59	80.96	0.424	0.52
	10	82.14	81.95	81.98	82.02	0.076	0.09
Average.....		81.42	81.51	81.60	81.51	0.187	0.23
60	1	81.65	81.65	81.59	81.63	0.027	0.03
	2	81.47	81.54	81.40	81.47	0.047	0.06
	3	80.54	81.12	80.92	80.86	0.216	0.27
	4	81.69	81.45	81.86	81.67	0.144	0.18
	5	80.24	80.20	80.12	80.19	0.044	0.05
	6	80.06	79.62	80.45	80.04	0.282	0.35
	7	80.41	79.72	79.88	80.00	0.269	0.34
	8	79.73	79.22	79.36	79.44	0.198	0.25
	9	80.68	80.72	81.54	80.98	0.373	0.46
	10	81.96	81.72	81.68	81.79	0.117	0.14
Average.....		80.84	80.70	80.88	80.81	0.172	0.21

thirty. Of the ten churnings, seven show a greater variability in water content at sixty revolutions than at thirty.

Table 3 shows that the distribution of salt in butter became less variable as the working process was prolonged. Churnings 1, 2, 4, 5, 6, 7, and 9 show a continuous decrease in variability throughout the working process. Churning 3 and 8 show greater variability at forty-five revolutions than at thirty, but less variability at sixty, while churning 10 shows less variability at forty-five and sixty revolutions than at thirty, but greater variability

TABLE 6

	30 REVOLUTIONS	45 REVOLUTIONS	60 REVOLUTIONS
Water.....	0.105	0.112	0.128
Salt.....	0.198	0.167	0.139
Curd.....	0.052	0.047	0.039
Fat.....	0.216	0.187	0.172

TABLE 7

	CHANGES IN MEANS OF AVERAGE DEVIATIONS FROM	
	30 to 45 revolutions	30 to 60 revolutions
Water.....	+0.007	+0.023
Salt.....	-0.031	-0.059
Curd.....	-0.005	-0.013
Algebraic sums.....	-0.029	-0.049
Fat.....	-0.029	-0.044

at sixty revolutions than at forty-five. Thus all ten churnings show a more even distribution of salt at 60 revolutions than at thirty, while eight of the ten churnings show a more even distribution at forty-five revolutions than at thirty.

Variation in the curd content of butter from different parts of the churn as affected by working is shown in table 4. Here it is noted that seven of the ten churnings show more even distribution of curd in the butter worked forty-five and sixty revolutions than in the butter worked thirty. Churnings 2, 4, and 9 show greater

variability at sixty than at thirty revolutions, while churnings 1, 7, and 2 show greater variability at forty-five revolutions than at thirty. The averages of the coefficients of variability, also of the average deviations, show less variability as the working process is prolonged.

Table 5 shows the variation in the fat content of butter taken from different parts of the churn as affected by working. It will be noted that the mean of the average deviations decreased 0.029 per cent from thirty to forty-five revolutions, and 0.015 per cent from forty-five to sixty revolutions. Small decreases are likewise noted in the respective coefficients of variability.

#### DISCUSSION

The variation in the composition of salted butter, from a commercial standpoint, is determined by the distribution of the three chief non-fatty constituents, namely; water, salt, and curd. Unevenness of curd, water and salt, or brine distribution results in variability in the fat content and lack of uniformity in the composition of the butter as a whole. This relation is shown by the comparison, of the algebraic sums of the changes in the means of the average deviations of the non-fatty constituents during the different stages of the working process, with the corresponding changes in the fat content. The means of the average deviations of the four constituents at the three stages of the working process as shown in tables 2, 3, 4, and 5, are given in table 6.

The changes in the means, of the four constituents from thirty to forty-five and sixty revolutions, with the algebraic sums of the changes of the non-fatty constituents are shown in table 7.

These figures show that the algebraic sums of the changes in the average deviations of the non-fatty constituents are approximately equal to the corresponding changes in the average deviations of the fat. Variability in the fat content of butter, therefore, expresses the variability in the composition of butter as a whole.

The composition of the butters reported in this paper, as expressed by the variability on the fat content, became less variable

as the working process was prolonged. The mean of the average deviations decreased from 0.216 per cent at thirty revolutions, to 0.187 at forty-five, and 0.172 at sixty. This reduction effected by thorough working is of commercial significance, in that, it demonstrates that insufficient working is one of the several factors that may cause a variation in the composition of butter coming from the same churning.

Of the three non-fatty constituents considered in this investigation, the curd was the most variable, the salt next, and the water least. On the other hand, the curd content had the least effect upon the variability in the composition of the butter as a whole, since it is present in such small amounts as compared with the water and salt. The salt content exerted the greatest influence upon the variability in the composition of the butter, and the water content the next greatest, as shown by their average deviations.

The variation in the water content is not as large as the variation shown in the results of some former investigations. Hunziker, Mills, and Spitzer (2) found as much as 2.94 per cent variation in water content of butter from different parts of the churn, and a variation of 1 per cent or more in the water content in ten out of eighteen churnings. The greatest variation found by the investigation reported in this paper was 0.55 per cent, while but two out of the ten churnings worked forty-five revolutions, and two out of ten churnings worked sixty revolutions, and none of the ten churnings worked thirty revolutions, showed variations greater than 0.5 per cent between different samples taken from the same churning.

The average of the coefficients of variability in the water content of the five smaller churnings, which ranged in size from 676 to 1288 pounds of cream, decreased from 0.75 per cent at thirty revolutions to 0.65 at sixty. The opposite results were shown by the five larger churnings which ranged in size from 1483 to 2257 pounds of cream. In these churnings, the variability increased from 0.72 per cent at thirty revolutions to 1.01 per cent at sixty. In four of these churnings, numbers 6, 8, 9, and 10, the variability

increased from thirty to forty-five revolutions and then decreased from forty-five to sixty revolutions, yet it remained greater at sixty than at thirty revolutions.

The decrease in variability in water content of the small churnings from thirty to sixty revolutions; and the increased variability of the larger churnings from thirty to forty-five revolutions, followed by a decrease at sixty revolutions, may be explained by the combined influences of salt and working upon the size of the water droplets present in the butter.

It is generally understood that, during the early stages of the working process of salted butter, there is a marked decrease in the number of small droplets and a decided increase in the number of the larger droplets. The working process may be continued, however, until there is a redivision of the water droplets resulting in the reduction of size to nearly the point that prevailed before the salting and working commenced.

The increase in size of the water droplets during the early stages of the working process results in a less even distribution and greater variability in water than at the stage before the salting and working was commenced. Prolongation of the working process from the early stages eventually reduces the size of the droplets and results in a more even distribution of the water and less variability. Thus in all churnings the variability of the water content increases to a certain point and then begins to decrease, approaching the original state of distribution before the salt was added and the working commenced. In the small churnings of this investigation the turning point was reached before the first observed stage of the working process namely, thirty revolutions. The comparison of the observations at thirty, forty-five, and sixty revolutions, therefore showed a decline in the variability in the water content as the working process was continued. On the other hand, in the large churnings the turning point was not reached until after the first observed stage of the working process. Thus, according to the observed stages, the variability in water content increased from thirty to forty-five revolutions and then decreased from forty-five to sixty revolutions, yet remained greater at sixty than at thirty revolutions.



The decrease in variability of the salt content as the working process was continued was to be expected. Being a foreign constituent of butter and added to it at, or near, the beginning of the working process, it is worked into the butter and dissolved by the water. Its distribution is naturally, extremely uneven during the early stages of the working process. Subsequent working, within practical limits, could not result in other than a more even distribution.

The curd content of butter is so small that ordinarily its variability in butter from different parts of the churn is of little concern from a commercial standpoint. Although a decrease was shown of the variability in curd as the working process was prolonged, in view of the relative crudeness of the method of analysis as compared with the slight changes noted, this change can be interpreted only as an indication.

#### SUMMARY

The effect of prolonged working upon the distribution of water, salt, curd, and fat in butter was studied. Samples of butter were taken from different locations within the churn at three stages of the working process, namely, thirty, forty-five, and sixty revolutions.

The composition of butter, as expressed by the variability in the fat content, became less variable as the working process was prolonged. Of the three non-fatty constituents, the curd had the least effect upon the variability in the composition of butter as a whole, while the salt exerted the greatest influence and the water the next greatest influence. The variability in the water content of small churnings decreased as the working process was prolonged; while in large churnings, an increase in variability was shown from thirty to forty-five revolutions followed by a decrease at sixty revolutions; however, the variability was greater at sixty revolutions than at thirty. A more even distribution of the salt and curd was obtained as the working process was prolonged.

## REFERENCES

- (1) LEE, C. E., HEPBURN, H. W., AND BARNHART, J. M.: A study of factors influencing the composition of butter. Illinois Agr. Exp. Sta. Bul. 137, 1909.
- (2) HUNZIKER, O. F., MILLS, H. C., AND SPITZER, GEORGE: Moisture control of butter. Purdue Univ. Agr. Exp. Sta. Bul. 160, 1912.
- (3) GUTHRIE, E. S., AND ROSS, H. E.: Distribution of moisture and salt in butter. Cornell Univ. Agr. Exp. Sta. Bul. 336, 1913.

## BUTTER VERSUS OLEOMARGARINE IN RICKET CONTROL IN PIGS\*

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The following report covers one year's experimental work in a coöperative experimental project between the Dairy Department and the Animal Husbandry of the West Virginia University and Experiment Station. This project is the more recent development of an earlier project started by these two departments in 1923 to study the vitamin A deficiency in white corn and to secure a comparative economic measurement of this deficiency by supplementing a white corn basal ration with butter, which was rich in vitamin A, and also oleomargarine commonly considered to contain lesser amounts of vitamin A. In the progress of this earlier project it was noted that when oats were fed in the basal white corn ration that the pigs did not develop rachitic symptoms at about 90 days as was commonly experienced in white corn feeding. Oats have not been previously classed by investigators as having either vitamin A, the growth promoting vitamin, or vitamin D, the anti-rachitic vitamin, in noticeable amounts. The same oat fed lots of pigs when fed butter and oleo failed in the ordinary feeding period of 90 to 100 days to show any rachitic symptoms.

This led to the development of a supplementary project in 1926, in which the basal ration used in 1923-1924-1925 was changed to a new basal ration, containing white corn, buckwheat middlings and tankage and the same mineral mixture as the original basal rations, but without oats being used. Butter and oleo were used as in the earlier project with both basal rations, 2 ounces being fed daily throughout the feeding period for each 100 pounds live weight. Table 1 shows the results of this trial.

This trial shows rather strikingly the fact that when oats were

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used in the ration that the ordinary rachitic symptoms did not develop during the ordinary feeding period of 112 days. It also further shows that when the basal ration did not contain oats that 50 per cent of each lot showed rachitic symptoms within 86 days after starting. The adding, however, of 2 ounces of butter daily per 100 pounds live weight of this same ration brought the lot

TABLE 1

*Ricket control experiment with pigs, West Virginia University and experiment station*

LOT NUMBER	DATE		NUMBER OF DAYS	NUMBER OF PIGS	AVERAGE INITIAL WEIGHT	AVERAGE FINAL WEIGHT	AVERAGE GAIN	AVERAGE DAILY GAIN	FEED PER 100 POUNDS GAIN	NUMBER OF PIGS GOING DOWN	NUMBER OF DAYS ON FEED BEFORE GOING DOWN
					pounds	pounds	pounds	pounds			
1	1926	Basal Ration I	112	4	38.5	182.5	144.0	1.29	405	0	—
2	1925	Basal Ration I with butter	98	5	46.4	169.2	122.8	1.25	387	0	—
3	1925	Basal Ration I with oleo	98	5	47.0	160.4	113.4	1.16	410	0	—
4	1926	Basal Ration II	112	4	39.3	184.7	145.4	1.29	396	2	86
5	1926	Basal Ration II with butter	112	4	39.0	197.0	158.0	1.41	386	0	—
6	1926	Basal Ration II with oleo	112	4	40.5	196.0	155.5	1.39	406	2	86

Basal Ration No. 1. White corn 500 pounds, oats 400 pounds, tankage 66 pounds, oil meal 33 pounds.

Basal Ration No. 2. White corn 300 pounds, buckwheat middlings 100 pounds, tankage 20 pounds.

Butter and oleomargarine fed 2 ounces daily per 100 pounds live weight.

Basal rations fed 5 pounds daily per 100 pounds live weight.

through 112 days feeding with no symptoms of rickets. The lot fed on the same basal ration, but with two ounces oleo daily per 100 pounds live weight, showed 50 per cent rachitic conditions within 86 days after starting.

Due to the fact that oats and butter have not been ordinarily considered to contain Vitamin D in large amounts, it was at first

questioned whether the rachitic symptoms observed were true rachitic symptoms or possibly confused with symptoms of *Vitamin A deficiency*. To the affected pigs in these lots, cod liver oil, both raw and oxygenated, was fed daily in amounts of 25 cc. per 100 pounds live weight. Although several of these pigs had developed well marked symptoms such as nervousness, dropping ears, off feed, wobbly walking, unable to rise, all pigs so treated, except one which died the first day of treatment, improved rapidly and were in normal market condition in 30 days after starting treatment. This seems to indicate rather conclusively that these symptoms were true rachitic ones and were held in check by either oats or butter added to the ration, but not when oleomargarine was used.

This project will be carried on again this year under the same plan, using basal ration I and II, and also using butter and oleo, but carrying all groups through breeding and farrowing to see whether the oats and butter serve as only a retarding influence on rachitic conditions or as a complete check throughout the life time feeding and activities of the pig.

It should be added that all lots were carefully balanced, being made up as near as possible from pigs from the same litter of the same age and conditions, and were given the same care and treatment throughout the entire experiment.

The oleomargarine was a nut margarine. The mineral mixture, composed of 5 pounds bone meal, 5 pounds acid phosphate, and 1 pound common salt, was uniform for all lots, and was fed at rate of 1 ounce per 100 pounds live weight daily. All lots of pigs were treated for worms before starting.

The Basal Ration I was composed of white corn 500 pounds, oats 400 pounds, tankage 66 pounds, oil meal 33 pounds, and fed 5 pounds daily per 100 pounds live weight.

The Basal Ration II was composed of white corn 300 pounds, buckwheat middlings 100 pounds, tankage 20 pounds, and fed 5 pounds daily per 100 pounds live weight.

All lots were fed and handled on comparable concrete floors and runways, and were in the sunshine. Photographs were taken of all lots at beginning and end of the trial.

# A COMPARISON OF THREE METHODS OF PASTEURIZING MILK FOR CHEDDAR CHEESE-MAKING\*

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Pasteurization of milk for use in the manufacture of cheese has been shown to be practicable and beneficial by Sammis and Bruhn (1912), Liska (1912), Stevenson (1923), Price (1927) and others. Various methods of pasteurizing were used by these workers. Since it is conceivable that different heat treatments of milk may affect the quality, yield and composition of the cheese produced, it is the purpose of this investigation to determine the effect on the cheese-making processes of three methods of pasteurization. These methods represent in a general way the type of heat treatments available for use in the manufacture of pasteurized milk cheese under commercial conditions.

## BACTERIOLOGICAL PROCEDURE

The number of bacteria in the milk examined in this work were determined on lactose agar plates which were incubated at 37°C. for forty-eight hours. The medium used for plating had the following composition:

Peptone (Difco).....	10 grams
Beef extract (Liebig).....	5 grams
Lactose (C.P.).....	10 grams
Agar agar.....	15 grams
Distilled water.....	1000 cc.

The reaction was adjusted to pH 7.0 and one lot only of the medium was used in making the counts from a single experiment.

Samples of milk for plating were held in cotton-stoppered, sterile test tubes which were packed in ice. The time interval between collecting and plating was never greater than four hours. Every dilution of each sample was plated in triplicate.

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## METHOD OF CHEESE MANUFACTURE

The milk used in each experiment was placed in a 300-gallon pasteurizer and thoroughly mixed. Four portions, weighing approximately 400 pounds each, were then removed while the milk was in motion. Each lot was sampled for bacterial counts at this time. Without any unnecessary delay these lots were subjected to the following procedures:

The first portion was placed in a cheese vat and held at approximately 60°F. until the three remaining lots were ready to make into cheese.

The second portion was pasteurized by heating to 160° to 165°F. in a flash heater and then cooled immediately by means of a surface cooler. This method of pasteurization will hereafter be called the "Flash" process.

The milk of the third portion was heated in a flash pasteurizer to 145° to 150°F. and then conducted to a cheese vat where it remained until the entire lot had been so treated. It was then cooled to about 88°F. by pumping it over a surface cooler. The average time of heating was approximately twenty-two minutes. Nine minutes elapsed before the milk was pumped over the cooler due to necessary changes in sanitary piping. The temperature of the milk was not permitted to fall below 142°F. before the cooling operation was started. The entire time of cooling averaged about eleven minutes. This method of pasteurization will be called the "Flash-Holder" process.<sup>1</sup> It was intended to duplicate approximately the holder method of pasteurization without the relatively expensive equipment of the usual holder method of treatment.

The remaining 400 pounds was pasteurized by the well known "Holder" method. The milk was heated in a batch pasteurizer to 145°F. and held at that temperature for thirty minutes after which it was cooled to approximately 88°F.

Exactly 300 pounds of each lot of milk was weighed into each of four 50-gallon jacketed cheese vats and samples were taken for

<sup>1</sup> This heat treatment was developed from a suggestion made by Mr. H. Feldmeir of D. H. Burrell and Company, Inc., Little Falls, New York.

bacterial counts. Clean flavored lactic starter was added immediately to the milk in amounts varying from 1 to 3 per cent. The starter was usually about eighteen hours old from the time of its inoculation. Each vat of milk was made into the best possible cheese by the procedure described by Price (1927).

This procedure was followed as closely as possible for 10 experiments carried on between March 2 and May 25, 1926. In one experiment an accident resulted in the loss of the raw milk. The other lots were made into cheese as usual. This fact can be observed in subsequent data in the non-agreement of the differences of the mean scores and the mean differences in score between the pairs of raw and pasteurized milk cheese.

The cheese were scored to determine their quality at the ages of four and eight months. Three or more judges<sup>2</sup> examined the cheese. The average of their scores was taken to represent the quality of the cheese. The estimates were made on the basis that a perfect score would be: flavor, 50; body and texture, 35; color, 15; finish, 10.

The cheese and whey were analyzed for fat and total solids by the Mojonnier method (Mojonnier and Troy, 1925).

#### BACTERIOLOGICAL RESULTS

The bacterial counts of the raw milk ranged from 1,100,000 to 190,000,000 per cubic centimeter. The percentage of the number of bacteria in the original milk which were destroyed by the different methods of pasteurizing are shown in table 1. The averages of the percentage reduction for the Flash, Flash-Holder and Holder heat treatments are 98.31, 98.65 and 98.96 respectively.

The Holder method is apparently the most effective in reducing the bacterial count, while the Flash method allows the greatest number of organisms to survive the treatment. It is interesting to note the reduction accomplished by the Flash-Holder process.

<sup>2</sup> The authors are grateful for the assistance of Mr. J. C. Marquardt of the New York Experiment Station (Geneva), Dr. E. S. Guthrie and Mr. W. Hochstrasser of the New York State College of Agriculture, and Mr. A. B. Hargrave of the New York State Department of Agriculture and Markets, in scoring the cheese.



Bacterial counts were made on the lots of milk immediately after leaving the Flash heater when it was at a temperature of 145° to 150°F. These determinations show that an average of 75 per cent of the total number of organisms in the raw milk are destroyed by this exposure. An additional 23.5 per cent of the number of bacteria in the original milk are eliminated during the subsequent holding period.

It is probably true that the addition of starter to pasteurized milk in the amounts used in cheese-making establishes imme-

TABLE 1  
*Percentage of bacteria in original milk destroyed by the three heat treatments*

EXPERIMENT NUMBER	FLASH	FLASH-HOLDER	HOLDER
1	98.91	99.35	99.09
2	99.83	99.86	99.76
3	99.83	99.77	99.89
4	99.60	97.72	99.13
5	96.48	96.80	98.00
6	99.55	99.21	98.79
7	99.89	99.91	99.93
8	96.19	97.80	98.24
9	97.90	98.42	98.37
10	94.91	97.71	98.40
Average.....	98.31	98.65	98.96

diately a predominance of organisms whose development inhibits the growth of those undesirable types which may have survived the heating process. It is therefore doubtful if the slight differences in the relative efficiencies of these heat treatments, as indicated by the percentage reduction of the numbers of organisms in table 1, are of any real significance in the manufacture of Cheddar cheese.

#### RESULTS OF CHEESE MANUFACTURE

##### *Effect of pasteurization on curdmaking*

The manufacture of cheese from milk pasteurized by any of these heat treatments is not unusual or difficult. The curd

resembles that obtained from very sweet raw milk of desirable quality. The only essential differences in the curd-making process are the slightly delayed coagulation in the flash heated milk and the slow acid development. The delayed coagulation can be corrected by using  $\frac{1}{2}$  to 1 ounce more of rennet or by setting at 88°F. instead of 86°F. as is customary. The acid development can be controlled by the addition of commercial starter.

The rate of coagulation of the four lots of milk is illustrated by the average Marshall rennet test before the addition of starter:

	<i>Spaces</i>
Flash.....	5.8
Flash-Holder.....	5.3
Holder.....	5.2
Raw.....	4.7

The difference between the rate of coagulation of the raw and pasteurized milk is probably due partially to increase in the acidity of the raw milk during the interval when the lots of heated milk were being subjected to their respective heat treatments. It is true however, that flash pasteurization causes a delay in the rennet coagulation

The curd produced from milk heated by the Flash method at 160° to 165°F. can be successfully handled in spite of the slower rate of coagulation. This observation of the curd-making process in these experiments is really unnecessary in view of the general use of the flash pasteurizer in New Zealand cheese factories (Stevenson, 1923) as well as in many factories in the United States. Cheddar cheese has also been made successfully on a commercial scale from milk pasteurized by the Holder method (Price, 1927). The curd produced by this heat treatment seems to resemble raw milk curd more closely than the curd produced by either of the other two methods of pasteurizing. The curd from the milk treated by the Flash-Holder process resembles that produced from the Holder-pasteurized-milk so closely that it is usually very difficult to distinguish between them.

*Effect of pasteurization on cheese quality*

Table 2 shows the mean scores of the cheese of the 10 experiments after four months of curing at 45° to 50°F. It is evident from an examination of these figures that pasteurization of milk by any of the three methods improves the quality of the cheese when it is partially cured. As might be expected, the improvement is chiefly in the flavor.

The results shown in table 3 are emphasized when Student's method (Student, 1908) is used to interpret the significance of the differences in the total score between the pairs of raw and

TABLE 2  
*Mean scores of the cheese when four months old*

	RAW	FLASH	FLASH-HOLDER	HOLDER
Flavor scores.....	36.88 $\pm$ 0.49	38.64 $\pm$ 0.26	38.40 $\pm$ 0.35	38.50 $\pm$ 0.27
Body and texture scores.	23.04 $\pm$ 0.15	23.25 $\pm$ 0.07	23.36 $\pm$ 0.07	23.34 $\pm$ 0.07
Total scores.....	84.61 $\pm$ 0.57	86.49 $\pm$ 0.42	86.62 $\pm$ 0.40	86.46 $\pm$ 0.45

TABLE 3  
*Mean gain in total scores of pasteurized milk cheese over raw milk checks at four months of age*

HEAT TREATMENT	GAIN IN SCORE	SIGNIFICANT ODDS
Flash.....	1.91	908 to 1
Flash-Holder.....	2.00	555 to 1
Holder.....	1.94	555 to 1

pasteurized milk cheese. The advantages of this treatment of this type of data are discussed by Love and Brunson (Love and Brunson, 1924). Love's tables (Love, 1924) have been used in conjunction with Student's formula.

From table 3 it is apparent that the improvement in the quality of the cheese due to pasteurizing by any of the three methods tried is practically the same when the cheese are four months of age.

After eight months in the curing room at temperatures ranging from 45° to 55°F. the quality of the cheese is indicated by the average scores of table 4. Here again, as at four months of age,

the flavor of the cheese shows the greatest improvement, due to the heat treatments to which the milk was subjected.

Table 5 shows the average gain in total score, at eight months of age, of the pasteurized milk cheese when compared with the raw milk cheese made from identical raw material. The significant odds are calculated by Student's formula.

Tables 4 and 5 indicate that the Flash treatment is not quite so

TABLE 4  
*Mean scores of the cheese when eight months old*

	RAW	FLASH	FLASH-HOLDER	HOLDER
Flavor scores.....	37.83 $\pm$ 0.37	39.26 $\pm$ 0.38	40.17 $\pm$ 0.38	40.01 $\pm$ 0.31
Body and texture scores.	23.10 $\pm$ 0.12	23.31 $\pm$ 0.07	23.51 $\pm$ 0.11	23.36 $\pm$ 0.11
Total scores.....	85.47 $\pm$ 0.37	87.16 $\pm$ 0.33	88.31 $\pm$ 0.43	88.01 $\pm$ 0.38

TABLE 5  
*Mean gain in total scores of pasteurized milk cheese over raw milk checks at eight months of age*

HEAT TREATMENT	GAIN IN SCORE	SIGNIFICANT ODDS
Flash.....	1.89	212 to 1
Flash-Holder.....	3.12	1999 to 1
Holder.....	2.70	4999 to 1

TABLE 6  
*Cheese from 100 pounds of milk and starter*

RAW	FLASH	FLASH-HOLDER	HOLDER
pounds	pounds	pounds	pounds
9.92 $\pm$ 0.16	10.18 $\pm$ 0.16	10.18 $\pm$ 0.16	10.32 $\pm$ 0.17

effective as the other two heat treatments in improving the quality of the cheese after aging for eight months. The Flash-Holder and Holder treatments show an even greater gain in score over the raw milk check than was apparent at four months of age. The difference in score between the cheese from Flash-Holder and Holder treated milk is too small to be of any significance.

It is apparent from these scores obtained from the cheese during the curing process that each method of pasteurization improves

the quality of the cheese, and that the Flash-Holder and Holder methods of treating the milk cause the greater improvement in quality during the life of the cheese.

### *Yield and composition*

The cheese was removed from the press on the day after it was made and weighed and sampled for fat and total solids tests. The average yield of the cheese is given in table 6.

TABLE 7  
*Average composition of the cheese*

MATERIAL	RAW	FLASH	FLASH-HOLDER	HOLDER
	per cent	per cent	per cent	per cent
Fat.....	34.37 $\pm$ 0.37	33.51 $\pm$ 0.25	33.19 $\pm$ 0.24	33.11 $\pm$ 0.30
Solids-not-fat.....	28.53 $\pm$ 0.22	28.30 $\pm$ 0.25	28.56 $\pm$ 0.30	28.55 $\pm$ 0.19
Moisture.....	36.88 $\pm$ 0.26	38.18 $\pm$ 0.37	38.24 $\pm$ 0.34	38.34 $\pm$ 0.32

TABLE 8  
*Fat, solids-not-fat, and moisture in the cheese*

MATERIAL	RAW	FLASH	FLASH-HOLDER	HOLDER
	pounds	pounds	pounds	pounds
Fat.....	3.41	3.40	3.37	3.41
Solids-not-fat.....	2.83	2.87	2.91	2.94
Moisture.....	3.67	3.90	3.90	3.97

TABLE 9  
*Average composition of the whey*

MATERIAL	RAW	FLASH	FLASH-HOLDER	HOLDER
	per cent	per cent	per cent	per cent
Fat.....	0.386 $\pm$ 0.018	0.344 $\pm$ 0.017	0.368 $\pm$ 0.017	0.370 $\pm$ 0.016
Milk-solids-not-fat..	6.43 $\pm$ 0.06	6.35 $\pm$ 0.07	6.37 $\pm$ 0.07	6.40 $\pm$ 0.06

The cheese made from the Flash and Flash-Holder treated milk show a gain of approximately 0.25 pound, which represents 2.5 per cent of the weight of the raw milk cheese obtained from the same amount of milk and starter. The Holder method heat treatment shows a gain in yield of 4 per cent. Such gains, which

are in harmony with the results of previous work (Sammis, 1912; Price, 1927), tend to offset the cost of processing the milk.

Analysis of the cheese brings out the fact that the increase in the yield of the pasteurized milk cheese is due largely to increase in its moisture content, as shown in table 7. This is further substantiated by the data of table 8 which shows the average pounds of fat, solids-not-fat, and moisture retained in the cheese from 100 pounds of milk and starter.

Analysis of the whey (table 9), at the time of dipping tends to demonstrate that in these experiments the increased yield of the pasteurized over the raw milk cheese is probably not due to the greater retention of fat and milk-solids-not-fat.

The differences noted in table 9 are too small to be considered significant for the number of observations. Other experiments (Sammis, 1912; Stevenson, 1923; Price, 1927) have shown that the slight tendency exhibited in these figures for the pasteurized milk cheese to lose less fat in the whey is to be expected under ordinary conditions.

#### APPLICATION OF THE RESULTS

The three methods of pasteurization, whose effect upon the process of Cheddar cheese manufacture are discussed here, suggest means by which any cheese factory can improve the quality of cheese produced. These treatments should prove especially valuable where milk of inferior quality must be made into cheese. The significant result of this work is the general improvement in the quality and yield of the cheese following the use of all the methods of pasteurizing. It is not intended to attempt to demonstrate here the other advantages which result from the pasteurization of milk for cheese making. The greater uniformity of the product and the improved keeping quality, as well as other facts, have been pointed out by those whose experiments and practical experiences with pasteurization have been previously mentioned in this discussion.

## SUMMARY

1. Milk pasteurized by any of the three heat treatments described produced higher scoring cheese than identical milk not pasteurized.

2. No calcium chloride or hydrochloric acid was necessary to stimulate rennet coagulation.

3. The Flash method of pasteurizing was not so effective as the other heat treatments.

4. The yield of cheese from pasteurized milk was increased from 2.5 to 4 per cent in excess of the yield obtained from identical milk not pasteurized, due to the retention of more moisture.

## REFERENCES

- BENSON, M., AND EVANS, R. H. 1913 The manufacture of cheese from "heated" milk. *Jour. Board Agriculture (Great Britain)*, xx, no. 4, 281-301.
- LISKA, A. 1912 Käsefabrikation aus pasteurisierter Milch. *Milchwirtschaftliches Zentralblatt*, xli, no. 16, 481-485.
- LOVE, H. H., AND BRUNSON, A. M. 1924 Students method for interpreting paired experiments. *Jour. Amer. Soc. Agron.*, xvi, no. 1.
- LOVE, H. H. 1924 A modification of Student's tables for use in interpreting experimental results. *Jour. Amer. Soc. Agron.*, xvi, no. 1, 68-73.
- MOJONNIER, T., AND TROY, H. C. 1925 The Technical Control of Dairy Products. Mojonnier Brothers Company, Chicago, Ill., pages 95-131.
- PRICE, W. V. 1927 Cheddar cheese from pasteurized milk. *Jour. Dairy Sci.*, x, no. 2, 155-168.
- SAMMIS, J. L., AND BRUHN, A. T. 1912 The manufacture of cheese from pasteurized milk. *Wisc. Expt. Station Res. Bul.* 27.
- STEVENSON, C. 1923 Pasteurization of milk for Cheddar cheese-making in New Zealand. *Proceedings of the World's Dairy Congress*, 1923, i, 306-308.
- "Student" 1908 The probable error of a mean. *Biometrika*, vi, 1-25.

# THE POSSIBLE TOXICITY OF BUTTERMILK SOURED IN ZINC CONTAINERS\*

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The general impression has long been held that zinc salts are toxic to man and animals. In the past there have been instances where foods, prepared or stored in zinc-lined vessels, were thought to have caused illness among persons consuming them.

In the spring of 1925, farmers in an Oklahoma community attributed a number of deaths among swine to the feeding of buttermilk held in a galvanized iron tank. They suspected that zinc dissolved from the lining of the tank, was the cause of the trouble. These facts, brought to the attention of the Oklahoma Agricultural Experiment Station, led to the following investigation to determine whether zinc had been the chief contributing factor.

## PREVIOUS INVESTIGATIONS

A review of the literature reveals interesting data regarding the possibility of zinc poisoning among men and animals.

Barnard and Bishop (1) found that lemonade stored in a zinc container dissolved an appreciable amount of zinc. The Indiana State Board of Health, under whose auspices this investigation was conducted, declared unlawful the further use of zinc-coated containers for the manufacture and storage of acid drinks.

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Seiffert (2) reported on metallic zinc poisoning as follows:

The damage due to zinc is underestimated. Zinc dust is swallowed and passes into the gastrointestinal canal. Continual, even though slight absorption of zinc, gradually produces illness. The greatest damage is done by the dusts of blende and calamine, which contain large amounts of zinc sulphate and zinc carbonate. Chronic zinc poisoning in workmen in zinc smelters cannot be denied. In fact it is the chief cause of the occupational disease of that industry.

Similar published reports are common.

Since this inquiry began, a series of publications of the Department of Physiology of the Harvard School of Public Health, leads one to question many previous investigations, and suggests that some of the earlier investigators may have erred in their conclusions, and that where poisoning occurred, it may possibly have been due to contamination with arsenic, lead or antimony off the galvanized surfaces rather than to the zinc.

Batchelor, Fehnel, Thomson and Drinker (3) have shown that men in contact with zinc metal suffered no disturbances as a result. Lutz (4) and Drinker and Collier (5) reported that zinc was a normal constituent of certain parts of the human body, was present in most foods, and in all probability performed a useful function in metabolism.

Recently we reported (6) that rats fed a complete ration to which zinc, or zinc salts, were added up to 0.25 per cent of the food consumed, suffered no unfavorable effects. Histological examination failed to reveal any pathological conditions. Analyses showed no appreciable increase in the content of zinc in the heart, liver, kidneys, spleen, lungs and testicles of these rats.

#### EXPERIMENTAL

The problem regarding the possible toxicity of buttermilk held in zinc storage vat, divides itself into two phases, analytical and biological; i.e., is the zinc content of the buttermilk increased through contact with zinc-lined vessels, and does such alteration render this buttermilk harmful to rats and swine?

In attempting to answer the first question, quantitative analy-

ses were made for the zinc content of cultured buttermilk soured in glass containers, in new, and in old zinc pails, held for various lengths of time, and at various temperatures. The zinc content of commercially churned buttermilk obtained from a creamery where it has been stored in a galvanized storage vat, was also determined.

#### METHOD OF ANALYSIS

The milk was placed in an evaporating dish and heated to boiling, then transferred to a vacuum oven until dry. It was then transferred to a muffle furnace and heated six hours at black heat. The black product resulting was extracted with hydrochloric acid

TABLE 1

*Effect of containers on the zinc content of commercial and cultured buttermilk*

SOURCE AND KIND OF SAMPLE	ZINC PER 1000 CC. OF SAMPLE	ACIDITY OF SAMPLE
	mgm.	per cent
Fresh milk.....	3.5	0.17
Buttermilk cultured in glass containers.....	3.5	0.80-0.9
Commercial buttermilk from old galvanized tank.....	7.0	0.80-0.9
Cultured buttermilk in new galvanized pail held twenty-four hours.....	150.0	0.80-0.9
Held four days .....	200.0	0.80-0.9

and filtered, and the process repeated, dampening with nitric acid until completely extracted. The acid filtrate was analyzed by the Birckner (7) method. This method, briefly stated, consists in adjusting the hydrogen ion concentration so that the solution when treated with hydrogen sulfide precipitates the zinc and leaves the iron in solution. The sulfide is well washed and dissolved in dilute hydrochloric acid. Aliquot parts of this solution were placed in Nesslerizing tubes and treated with potassium ferrocyanide. The resulting turbidity was compared with a series of standard zinc solutions, simultaneously prepared and treated.

Better results were obtained by first removing the iron by the basic acetate method and then proceeding as directed. This step overcomes the difficulty of completely removing all the iron, as

was recommended in the original Birckner method. The results of the analyses confirmed the report of Birckner (7) that normal milk contains zinc. It further demonstrated that the milk dissolved additional zinc from the container, as an inspection of the surface had indicated previously. Further, it was found that the zinc content increased with length of time of storage, with increased temperatures, with the acidity of the buttermilk, and with newness of the containers. It is probably that some of these effects overlap in results reported here. An average set of analyses illustrating these facts, is given in table 1.

#### BIOLOGICAL TESTS WITH RATS

Rats and swine were used as experimental animals. The rats being used in larger numbers served as a check and also to verify the effects obtained on a very limited number of swine. Albino rats were chosen from the stock colony of the department of agricultural Chemical Research, the normal growth curve, age of reproduction, and size of litters of which had been established. They were fed the following basal ration:

Yellow corn, ground.....	50
Whole wheat, ground.....	41
Tankage.....	5
Sodium chloride.....	1
Calcium carbonate.....	1
Cod liver oil.....	2

The rats were divided into six lots, with respect to age, size and parentage, and placed in round metal cages. The basal ration was supplemented with buttermilk and zinc salts as shown in table 2.

Reports in the literature led us to expect deleterious effects with all rations containing zinc. Our experimental animals showed few serious effects from these rations. Details concerning relative growth and reproduction are summarized briefly in table 3.

It is observed that Lot II receiving buttermilk at will, grew better and had larger litters than those receiving the basal ration only. Lot III likewise grew above the normal rate, produced

young, and even the second and third generations produced young without exhibiting any deleterious effects from the ration employed. Lot V also made fair growth, and reproduced, though a larger proportion of their offspring died. Lot VI made poor growth, and failed to reproduce. After several weeks they showed loss of appetite, the hair became rough, and they suffered

TABLE 2  
*Experimental rations received by rats*

LOT	SUPPLEMENT RECEIVED IN ADDITION TO BASAL RATION
I	None
II	Buttermilk soured in glass, ad libitum
III	Buttermilk soured in new zinc pail, ad libitum
IV	Buttermilk from old galvanized tank, ad libitum
V	0.5 per cent of ration as zinc lactate
VI	1.0 per cent of ration as zinc carbonate

TABLE 3

LOT	SUPPLEMENT	MALES	FE- MALES	LITTERS YOUNG	YOUNG LIVED	YOUNG DIED	GROWTH
I	None	2	2	3	18	3	+++++
II	Buttermilk soured in glass	2	2	3	23	2	+++++
III	Buttermilk soured in new zinc pails	4	6	7	48	3	+++++
IV	Buttermilk from old zinc tank	2	2	4	28	1	+++++
V	0.5 per cent zinc lactate	2	2	2	9	4	+++++
VI	1.0 per cent zinc carbonate	2	3	0	0	0	++

from diarrhea. They were retained far beyond the period of sexual maturity, but did not reproduce.

These results lead one to conclude that the amounts of zinc ordinarily found in food contamination, may have little or no harmful effects. It is only when the zinc salt is increased to abnormal amounts that growth and reproduction are affected. Such amounts are seldom found in food under ordinary conditions.

## BIOLOGICAL TESTS WITH SWINE

Eight Hampshire gilts of similar breeding, each weighting approximately 100 pounds, were obtained by the Dairy Department for use in a feeding trial. These animals were divided into four lots of two animals each, and placed in four separate pens on February 1, 1926. Each pen was 12 by 14 feet, and was provided with a concrete runway 12 by 16 feet which gave the animals access to sunlight and permitted some exercise. Wheat straw was used as bedding, and was replaced as needed. The following spring, they were moved to more spacious quarters, having access to unsurfaced exercise lots.

TABLE 4  
*Rations consumed by experimental swine*

LOT	NUMBER OF GILTS	SUPPLEMENTS ADDED TO BASAL RATION
I	2	Tankage from February 1 to April 12, 1926. On April 13, buttermilk containing 0.5 to 1.0 per cent of commercial zinc carbonate, was fed. Zinc carbonate was discontinued on May 11, 1926
II	2	Plain buttermilk
III	2	Buttermilk from a galvanized iron tank in a commercial creamery
IV	2	Cultured buttermilk held from twelve to forty-eight hours in new galvanized iron pails

The basal ration fed to all lots, consisted of yellow corn and alfalfa hay, varied in amount according to appetite, rate of growth, and general thriftiness. Fresh water and salt were always available. Some fresh grass was fed in season, replacing the alfalfa hay.

In addition to the basal ration, each lot received buttermilk, or tankage, or tankage supplemented with zinc salts, as shown in table 4.

The feeding trial began on February 1, 1926 and continued until after farrowing with all animals except those in Lot I, which were discontinued on September 13, 1926.

## DISCUSSION OF RESULTS

*Pen I*

The two gilts in Pen I constituted the control lot. They received yellow corn, alfalfa hay and tankage in addition to salt and water. They made normal gains, as would be expected. On April 13, 1926, tankage was withdrawn, and replaced with buttermilk to which 0.5 to 1.0 per cent of commercial zinc carbonate had been added. For two weeks they drank this product with apparent relish. It was then noticed they began to prefer water to the treated buttermilk. Shortly thereafter, the smaller gilt exhibited increasing stiffness of the hind legs. Whether this was due to the zinc carbonate to lack of exercise, to a shortage of minerals in the diet, or to a rheumatic condition caused by being continually on concrete floors, was undetermined. However, it is well to note that repeated veterinary examinations failed to disclose definite symptoms of zinc poisoning. Zinc carbonate was withdrawn from the ration on May 11, 1926, and the animal permitted free access to grass and to bone phosphate, while cod liver oil was added to her ration. For awhile she appeared less animated, and seemed somewhat in pain, but continued to show slow gains in weight. When returned to the Animal Husbandry department on September 13, 1926, this animal still exhibited some stiffness of the hind legs. The pen-mate was unaffected. Both animals were pregnant. It is therefore not apparent whether zinc carbonate played any part in the observed pathological condition.

*Pen II*

The gilts in Pen II received the basal ration plus plain buttermilk. This buttermilk was the product made from churning cream that had been neutralized to approximately 0.3 per cent acidity at the churn, or was cultured in tinned containers from separator-skimmed milk. Both gilts made satisfactory gains in weight. On September 4, 1926, the first gilt farrowed one dead and four living pigs, healthy and normal. The second gilt was bred repeatedly, finally farrowing one dead and nine live pigs on July 22, 1927. These were of good size and active.

*Pen III*

The gilts in Pen III received the basal ration plus buttermilk obtained from the churning of neutralized cream in a commercial creamery. This buttermilk was pumped from the churn directly to a large galvanized iron storage tank and held twelve to twenty-four hours before being drawn off by creamery patrons and taken to the farms there to be fed to hogs. Through the courtesy of the creamery, regular shipments of this buttermilk were obtained for use in the feeding trial.

TABLE 5  
*Record of farrowing of experimental swine*

LOT	SUPPLEMENT USED (CONTROL SOWS LATER RECEIVING)	SOW	DATE OF FARROWING	NUMBER OF PIGS		AVERAGE WEIGHT OF PIGS	WEIGHT OF SOW AFTER FARROWING
				Live	Dead		
						pounds	pounds
I	Zinc carbonate as in table 4	1	Pregnant	Withdrawn September 13, 1926			327
		2	Pregnant				276
II	Plain buttermilk	3	September 4, 1926	4	1	2.29	328
		4	July 22, 1927	9	1	2.78	434
III	Buttermilk from zinc storage vat	5	February 25, 1927	10	1	2.67	419
		6	September 2, 1927	6	0	2.70	537
IV	Buttermilk soured in galvanized pails	7	December 21, 1926	9	0	3.39	449
		8	May 31, 1927	5	0	3.47	480

Both gilts came in heat normally, and were bred. On February 25, 1927, the first sow farrowed one dead and ten live pigs that appeared normal but were somewhat weak. The second sow was served seven times. She finally became pregnant, and farrowed six live pigs on September 2, 1927.

*Pen IV*

Two pigs in Pen IV received the basal ration plus buttermilk soured in galvanized iron pails of 14 quart capacity. This butter-

milk was made by allowing skim milk to stand at room temperature for twelve to forty-eight hours. New pails were purchased and used as frequently as the zinc coating appeared to be dissolved from the inner surface of the pail. Both gilts came in heat normally and were bred. On December 21, 1926, the first sow farrowed nine live, healthy pigs, all of good size except one. The second sow, after repeated breeding, conceived and farrowed five healthy pigs on May 31, 1927.

Since data were desired only as to the effect of the zinc in the buttermilk upon the sows themselves, and upon their ability to reproduce, each animal was withdrawn from the trial immediately after farrowing. No indication was noted that the zinc dissolved from zinc-lined containers by buttermilk, had any effect upon the sows themselves, nor upon the size and vigor of the offspring. The delay experienced in getting some of the animals to conceive, may or may not have been due to the degree of fatness of the animals at time of breeding. It appears that such may have been the same, since this difficulty was experienced with one animal in each of the last three lots.

#### DISCUSSION OF RESULTS

Several factors are worthy of note before any conclusions are drawn. In the first place, we acknowledge that our facilities were too limited to permit a larger number of swine to be used in this phase of the work, but believe that the data obtained from them, together with that secured from the feeding trials with rats, are sufficient to be of value in throwing some light upon the possible toxicity of the buttermilk held in zinc-coated equipment, and of the zinc compounds used.

It was necessary to re-breed several of the gilts, one as many as seven times, before they became pregnant. Doubtless the boars were not at fault, since they rendered satisfactory service with other animals in the regular college Hampshire herd. Since as much difficulty was experienced with Lots II and III as with Lot IV which received the buttermilk containing the largest amounts of zinc, as shown in table 1, it is thought by the authors that the failure to conceive readily was not caused by the zinc, but



possibly by the excessively high fat condition of the animals receiving such a good ration, or to a lack of sufficient exercise. The feeding trial was begun with gilts weighing approximately 100 pounds. As far as health and reproduction are concerned, the buttermilk soured in zinc containers appeared to have no marked effect, *with animals of this size*.

Attention should also be called to the fact that the gilts in Lots I and IV were fed buttermilk containing dissolved zinc in much greater quantities than would ever be given under ordinary conditions. Therefore, in the light of the results obtained with both rats and swine, the following conclusions seem warranted.

#### CONCLUSIONS

1. Buttermilk dissolves the zinc from the surface of galvanized vessels in proportion to (a) acidity of the buttermilk, (b) area of surface exposed per unit of buttermilk present, and (c) time of exposure.

2. Small amounts of zinc lactate such as might be found in food appear to have no marked effect upon growth and reproduction of rats and swine.

3. A relatively large amount of zinc carbonate (1 per cent or more) appears to retard growth and reproduction of rats. The exact nature of the injury is not entirely evident.

4. It would appear that buttermilk soured in galvanized containers has no deleterious effect upon growth and reproduction of rats and swine, under the conditions of this experiment.

#### REFERENCES

- (1) BARNARD, H. E., AND BISHOP, H. E.: Indiana State Board of Health Report, 1908, 254.
- (2) SEIFFERT: Chem. Zentr., 1918, ii, 305; Chem. Abstr., 1920, xiv, 980.
- (3) BATCHELOR, R. G., FEHNEL, J. W., THOMPSON, R. M., AND DRINKER, K. R.: Jour. Ind. Hyg., 1926, viii, 322.
- (4) LUTZ, R. E.: Jour. Ind. Hyg., 1926, viii, 177.
- (5) DRINKER, K. R., AND COLLIER, E. S.: Jour. Ind. Hyg., 1926, viii, 257.
- (6) HELLER, V. G., AND BURKE, A. D.: Jour. Biol. Chem., 1927, lxxiv, 85.
- (7) BIRCKNER, V.: Jour. Biol. Chem., 1919, xxxviii, 191.

# INFLUENCE OF TEMPERATURE ON THE CHANGES PRODUCED IN MILK BY CERTAIN BACTERIA\*

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## INTRODUCTION

The changes brought about in various materials by bacteria are extremely important in the study of these organisms. Certain of them are investigated chemically in an attempt to explain the source of various products formed, while those that can be readily detected are widely used in the identification of organisms.

Milk may undergo a variety of changes because of the number of constituents it contains which are easily broken down; some of these changes, especially those in the casein, are very conspicuous. Milk is, therefore, a valuable medium for use as an aid in identifying certain organisms and is widely employed for this purpose.

Observations made at various times indicated that with certain organisms the temperature at which growth occurred had an influence on the type of change brought about in milk. An attempt was accordingly made to determine with what organisms there was such an effect, and also whether or not it was pronounced enough to be given consideration; the results secured are reported herein.

## GENERAL PROCEDURE

In the study of the influence of temperature on the changes brought about in milk by bacteria the only temperatures used were room temperature and 37°C. Tubes of milk from the same lot were inoculated, using approximately equal amounts of inoculating material, and some held at room temperature and the others at 37°C.; observations on the changes occurring were then made at various intervals over a rather extended period. The

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rate of change at the two temperatures was also studied in a general way by means of acid and soluble nitrogen determinations and some attention was given to the numbers of organisms present.

#### METHODS

The lots of milk studied were held in test tubes in quantities of about 10 cc. When a given determination was run on different days new tubes were used each time.

*Acid determinations.* The acid determinations were made by titrating a measured portion of the milk, usually 5 cc., with N/20 NaOH using phenolphthalein, and calculating as lactic acid.

*Soluble nitrogen determinations.* The soluble nitrogen was separated by measuring a small volume of the milk, usually 5 cc., into a beaker, adding water and a few drops of glacial acetic acid and then filtering. Nitrogen determinations were run by the Kjeldahl method on both the soluble and insoluble portions, so as to provide data for a check on the total amount of nitrogen.

*Bacterial counts.* The bacterial counts were run by the plate method. Whey agar was used for the true lactic acid organisms and the standard agar for milk analysis for the other types; the incubation was forty-eight hours at 37°C. in all cases.

*Sampling.* Before sampling flocculated or curdled material for various purposes it was broken up and distributed as uniformly as possible by means of a sterile glass rod.

#### RESULTS OBTAINED

The organisms studied may conveniently be divided into two groups, (1) those belonging to the lactic acid bacteria and attacking principally the lactose, and, (2) those belonging to the proteolytic types and having their conspicuous action on the casein. The former may be separated into (a) the true lactic acid organisms and (b) the gas forming lactic acid organisms.

##### 1. *Lactic acid bacteria*

a. *True lactic acid organisms.* There was no evidence that temperature had an effect on the type of the change produced in

milk by the true lactic acid bacteria but certain of these were nevertheless investigated.

*Streptococcus lactis*. Two typical *S. lactis* cultures were studied, and with both of them there was no observable difference in the type of change produced in litmus milk at room temperature and at 37°C. The reduction, coagulation and return of the color proceeded in essentially the same manner in both cases. At 37°C. definite reduction of the litmus and the subsequent changes occurred much more quickly than at room temperature, as is usually the case with *S. lactis* cultures, although there are certain ones with which the changes at 37°C. are slower than at room temperature and some with which there is no growth at 37°C. Covering the surface of the milk with nujol to limit the oxygen supply had no definite influence on the changes produced in milk by the organisms.

Determinations of the acidity at various intervals showed that, with the cultures studied, acid was produced much more rapidly at 37°C. than at room temperature during the early part of the holding period, but that later the acidity was higher at room temperature. The differences between the acidities eventually developed at the two temperatures were not large but were obtained regularly. This is in agreement with unreported data previously secured at the Iowa Agricultural Experiment Station with a larger number of *S. lactis* cultures. It would be expected that with the organisms growing so well at the two temperatures, the acid developed would have more of an inhibiting effect at the higher temperature. No increase in the amount of soluble nitrogen in milk in which the organisms had grown was noted at either temperature.

Bacterial counts showed that the decrease in the number of organisms following the increase during the period of active growth occurred more quickly at 37°C. than at room temperature.

*Lactobacillus bulgaricus*. The *L. bulgaricus* culture studied grew very slowly in litmus milk at room temperature and very rapidly at 37°C. so that the variation in the effect of these two temperatures on the rate of change with this organism was pronounced. The general character of the changes at the two

temperatures was the same, however, and cultures at room temperature had the appearance of cultures in the same stage of development at 37°C. When the surface of the milk was covered with nujol, growth was essentially the same as when the oxygen supply was not limited.

The total acidity produced, even after a number of days, was always much lower at room temperature than at 37°C. No increase was noted at either temperature in the soluble nitrogen in the milk in which the organism had grown.

The decrease in the number of organisms following the active increase occurred more quickly at 37°C. than at room temperature.

*Lactobacillus casei*. The *L. casei* culture studied grew much less rapidly at room temperature than at 37°C., but the changes in litmus milk were essentially the same at the two temperatures, and involved reddening and reduction of the litmus, and coagulation. Flooding the surface of the milk with nujol had no definite effect on the rate or type of change produced in milk by *L. casei*.

The total acidity developed in milk, even after six days incubation, was considerably higher at 37°C. than at room temperature; at the former temperature the acid production was especially rapid during the first two days. No increase in the soluble nitrogen was noted as a result of the growth of the organism in the milk.

The bacterial counts showed that both the increase and the decrease following it were more rapid at 37°C. than at room temperature.

*b. Gas forming lactic acid organisms*. The preliminary observations suggested that temperature had only a slight effect on the changes produced in milk by the gas forming lactic acid organisms; however, two species belonging to this group were studied.

*Aerobacter aerogenes*. The culture of *A. aerogenes* used was isolated from the mouth of a cow. At room temperature sliminess developed in litmus milk in from six to seven hours and increased with age; the litmus was soon reddened. Gas bubbles were noted and were greatly increased in numbers when the tubes were jarred. The slimy material became very conspicuous

at the surface and appeared as a definite layer, while beneath it the casein seemed to separate out slightly. Later the sliminess decreased and there was a reduction of the litmus which gave the cultures a white appearance. At 37°C. growth occurred more quickly, as judged by the development of sliminess and the reddening of the litmus. Gas was soon evident also. There seemed to be a more definite separation of the casein than at room temperature and sometimes even the suggestion of a firm curd. Reduction of the litmus occurred sooner than at room temperature but the sliminess was never so pronounced. Covering the surface of the litmus milk with nujol to limit the air supply seemed to slow up the changes at both temperatures but did not influence their general character.

Although the results were not uniform, in general acid was produced in milk more rapidly and in larger amounts at 37°C. than at room temperature, while at both temperatures the limiting of the oxygen supply favored acid production. The soluble nitrogen values found showed that there was no definite change in the amount of soluble nitrogen in milk in which the organism had grown.

The bacterial counts indicated that the decrease in the number of organisms following the increase occurred sooner at 37°C. than at room temperature.

In general growth seemed to be more rapid at 37°C. than at room temperature and there was more of a tendency toward the formation of a definite curd. Sliminess seemed to be favored by the lower temperature but was pronounced at both.

*Aerobacter oxytocom.* The culture of *A. oxytocom* used was isolated from a sample of gassy cheddar cheese. At room temperature gas formation and reddening of the litmus were evident in litmus milk in about twelve hours, while after twenty-four hours gas was present in large amounts. Reduction of the litmus began after four or five days and was almost complete in from seven to ten days. Usually there was only a suggestion of coagulation and no definite curd was formed. Often there was some development of sliminess. At 37°C. gas formation and reddening and reduction of the litmus occurred more quickly than at room tem-

perature. Later coagulation took place and was much more definite than at room temperature; the curd often showed fissures through which gas had escaped. With age the curd became tough and rubbery. Sliminess was sometimes noted but was not as pronounced as at room temperature. Limiting the oxygen supply by covering the surface of the milk with nujol seemed to decrease the rate at which the organism brought about changes at either temperature.

Determinations showed that acid production started off more rapidly at 37°C. than at room temperature but that the higher acidities were reached at the latter temperature. The use of nujol at the surface of the cultures had no definite effect on the acid production. No change was noted at either temperature in the soluble nitrogen in milk in which the organism had grown.

Bacterial counts indicated that the decrease in the numbers of organisms following the increase during active growth occurred more quickly at 37°C. than at room temperature.

The principal difference in the changes in milk at room temperature and at 37°C. was in the coagulation, a definite curd being formed at the latter temperature but not at the former. There was an abundance of gas at both temperatures but the sliminess was more pronounced at room temperature.

## 2. *Proteolytic types*

The preliminary observations suggested that temperature had a rather definite effect on the type of change produced in milk by some of the proteolytic organisms and accordingly a number of these were studied.

*Staphylococcus cremoris-viscosi*. The culture of Staph. cremoris-viscosi studied came originally from an outbreak of ropy milk on an Iowa farm.<sup>1</sup> In litmus milk at room temperature ropiness was evident in from seven to nine hours and increased with age. After about sixteen hours a clear layer of viscous material developed at the surface and then later there was a suggestion of a flocculation of the casein. The precipitated casein soon began to

<sup>1</sup> Jour. Dairy Sci., iii, 291, 1920.

digest near the surface. The flocculation and digestion continued until there was a distinct layer of curd in the bottom with a clear liquid above. With the appearance of a definite change in the casein the ropiness disappeared entirely. At 37°C. milk cultures became ropy in from six to ten hours. After about twenty-four hours there was definite coagulation with a thin film of clear slightly ropy material at the surface. The increase in the depth of the clear surface portion suggested digestion; the clear portion soon lost its viscosity. A considerable mass of curd remained in the lower portion of the tubes even after very long holding periods. Often the curd remained attached to one side of the tube, as it decreased in amount, instead of collecting at the bottom. Limiting the oxygen supply by flooding the milk with nujol greatly decreased the rate of change in litmus milk at both temperatures but had comparatively little influence on the type of change.

Acid determinations were made at frequent intervals on cultures grown at room temperature and at 37°C., both with and without nujol. The results showed that acid development was lower at room temperature than at 37°C. and also slower under nujol than when exposed. In no case, however, was the acidity sufficient to cause coagulation. Comparisons were made of the soluble nitrogen in cultures grown at room temperature and at 37°C. for varying periods and a greater amount was regularly found in the room temperature cultures. This agrees with the general appearance of the cultures developed at the two temperatures.

Bacterial counts showed that the organisms reached their maximum numbers much more quickly at 37°C. than at room temperature and that after several days there was a pronounced decrease at 37°C., while at room temperature the decrease was only slight.

The principal difference in the action of the organism on milk at the two temperatures was a more conspicuous digestion at room temperature and a much more definite curd formation at 37°C. It was found that the temperature effect was definite and independent of the age of the inoculating material or the temperature at which it had been grown.



*Pseudomonas fluorescens*. At room temperature litmus milk cultures showed no change during the first twenty-four hours but during the next twenty-four hours there was a definite clearing at the surface. The digestion increased with age from the surface downward and there was some settling out of the casein but this material also digested and eventually the entire material was quite clear. The change in the color of the litmus suggested a decrease in the acidity, and there was some reduction beginning at the bottom. Usually a dry pellicle developed at the surface of the cultures. At 37°C. digestion was evident at the surface in twenty-four hours, while in the lower portions of the tubes there was some flocculent coagulation. Digestion continued and involved this flocculent material, although there remained a small amount of curd in the bottoms of the tubes even after six or seven days. By limiting the oxygen supply with a layer of nujol the rate of digestion was greatly decreased at both temperatures.

Determinations showed that acid production in milk was somewhat slower at room temperature than at 37°C. and that it was slower at both temperatures when the milk was covered with nujol than when it was exposed. In all cases, however, the acidity remained very low. Comparisons of the soluble nitrogen in cultures grown at room temperature and at 37°C. for varying lengths of time showed a tendency toward more soluble nitrogen at 37°C. early in the holding period, while later the differences were negligible. This is in agreement with the observations that digestion was pronounced at both temperatures, although it started off more rapidly at 37°C.

Bacterial counts showed the same general numbers of organisms at the two temperatures.

With *Ps. fluorescens* the conspicuous change in milk at either room temperature or 37°C. was a digestion. Coagulation was rather indefinite but at 37°C. there was more of a tendency toward a flocculation of the casein and a settling out of a mass of curd than at room temperature.

An enzyme preparation was made by transferring some of the clear digested material from a milk culture grown eight to ten days at room temperature to a sterile test tube, adding  $\text{CHCl}_3$ ,

and stoppering the tube; the mixture was frequently shaken during a holding period of several hours at room temperature. When this preparation was added to sterile milk in the ratio of one to ten, it caused a digestion at either temperature, without flocculation or reduction of the litmus; after six or seven days digestion was practically complete. Both microscopic examinations and transfers to agar slopes failed to show the presence of organisms.

*Proteus vulgaris*. With the culture studied growth at room temperature was comparatively slow. Digestion began at the surface after several days and progressed downward; when digestion was almost complete a small amount of material had settled out. The color of litmus milk changed to a yellowish brown which darkened with age. At 37°C. reduction of the litmus began in about twenty-four hours and in about thirty hours there was a layer of digested material at the surface. Beneath the digested area the milk developed a slight flocculation of the casein. Digestion of this material took place until there was only a slight sediment and the milk had a light brown color. When the oxygen supply was limited by covering the surface of the milk with a layer of nujol, the rate of growth was decreased at both temperatures, but there was no influence on the type of change.

Acid determinations at various intervals on milk in which the organism was growing showed a slight increase in acid and then a decrease. In general the increase was less and the decrease went to a lower point at 37°C. than at room temperature. Flooding with nujol resulted in a higher acidity and a smaller decrease at both temperatures. Determinations of the soluble nitrogen after various growth periods showed that an increase began more quickly and was slightly more pronounced at 37°C. than at room temperature.

Bacterial counts indicated no very striking differences in the numbers of organisms at the two temperatures during the period of examination.

With *P. vulgaris* as with *Ps. fluorescens* coagulation was not conspicuous at either room temperature or 37°C. but there was more of a tendency to a flocculation of the casein at the latter temperature than at the former.

*Bac. ichthyosmius*.<sup>2</sup> The culture used was isolated from a can of fishy evaporated milk.<sup>3</sup> At room temperature there was a very soft curd and a fishy odor in litmus milk in about twenty-four hours. Soon a digested layer appeared at the surface and this deepened with age until after six or seven days there was only a small mass of sedimented curd and this slowly decreased with continued incubation. The fishy odor was pronounced throughout the holding period. At 37°C. there was a definite but rather soft curd in twenty-four hours. The curd showed some tendency to whey off and to shrink but even in old cultures the lower half of each tube was still filled with a mass of curd. The fishy odor seemed to be less pronounced than at room temperature. A very few gas bubbles were noted at both room temperature and 37°C. Limiting the oxygen supply by covering inoculated milk with nujol resulted in a slower change at both temperatures.

Determinations at various intervals showed that acid production was more rapid and reached higher values at room temperature than at 37°C., although the final acidities were low in either case. When nujol was used to limit the oxygen supply, the acid development was always slower. The increase in the soluble nitrogen was more rapid and reached higher values at room temperature than at 37°C.; this is what would be expected from the appearance of milk cultures at the two temperatures.

The decrease in the numbers of organisms following the increase apparently began sooner at 37°C. than at room temperature.

The striking difference between the cultures of *Bac. ichthyosmius* at the two temperatures was the more definite coagulation and less complete digestion at 37°C. than at room temperature.

An enzyme preparation was made by the method used with *Ps. fluorescens* and when added to milk it caused a digestion without definite curd formation in about three days at either temperature. Microscopic examinations and transfers to agar slopes showed that the digested milk was sterile.

<sup>2</sup> Although incorrect, the original designation is retained. Bergey's designation, *Escherichia ichthyosmia*, is evidently also incorrect. The organism probably belongs to the genus *Proteus*.

<sup>3</sup> Agr. Expt. Sta., Iowa State Col. Res. Bul. 38, 1917.

## TRIALS WITH ADDITIONAL ENZYME PREPARATIONS

A number of attempts were made to secure enzyme preparations from various proteolytic organisms that produced rapid changes in milk. The usual procedure followed was to grow milk cultures for several days, add an excess of  $\text{CHCl}_3$ , cork tightly and then shake frequently. The clear liquid collecting at the top was tried out for its effect on sterile milk in the proportion of one to ten, usually at both room temperature and  $37^\circ\text{C}$ . The organisms used were two cultures of *Staph. cremoris-viscosi*, two of *Bac. ichthyosmii*, one of *Ps. fluorescens* and one of *Strep. liquefaciens*, and a number of preparations were made with each. Agar slope cultures were regularly used to establish the sterility of the mixtures of milk and the enzyme materials.

The preparations secured with the two cultures of *Staph. cremoris-viscosi* and the one culture of *Strep. liquefaciens* all failed to produce a change when added to sterile milk. Those secured with the two cultures of *Bac. ichthyosmii* and the one culture of *Ps. fluorescens* were quite active.

With the enzyme preparations made from the cultures of *Bac. ichthyosmii* the general change in sterile milk was a digestion and coagulation. In general the action was more rapid and the coagulation more conspicuous at  $37^\circ\text{C}$ . than at room temperature, although the difference in the changes produced was not striking and was not as pronounced as the difference between the changes secured when *Bac. ichthyosmii* was grown in milk at the two temperatures. About six hours was the shortest time in which a definite change was noted in the appearance of the milk to which one of the preparations had been added.

The enzyme preparations secured from the culture of *Ps. fluorescens* also caused a coagulation and digestion in sterile milk; they seemed to be quite active at  $37^\circ\text{C}$ . but at room temperature did not produce a definite change. In general coagulation seemed to be more conspicuous than in milk in which the organism was growing. About two days was the shortest period in which a definite change was produced.

After holding about sixteen months all the preparations seemed to be less active than when they were first prepared.

## DISCUSSION OF RESULTS

From the results given it is evident that with certain organisms the type of change produced in milk at room temperature may be different than that produced at 37°C. The greatest difference noted was with certain of the proteolytic organisms and involved the formation of a more definite curd at 37°C. than at room temperature; there was also a difference with the gas forming lactic acid organisms but with the true lactic acid forms the type of change was not influenced by the temperature, although the rate was. This effect of temperature is of importance from the standpoint of the descriptions of organisms and suggests that in giving the changes produced in milk the temperature should be stated.

It seems probable that because of its complexity milk might show a greater difference in the changes produced at various temperatures than most media. However, certain results indicate a pronounced temperature effect in the changes occurring in other media; for example, Hammer<sup>4</sup> found that *Bac. ichthyosmii* produced acid in salicin bouillon at room temperature, while at 37°C. an alkaline reaction developed.

There are various possibilities in connection with the cause of a difference in the changes produced at different temperatures. One explanation is a variation in the effect of the products of bacterial growth. This seems a probability with such organisms as the aerobacter types studied, where the acid is just about enough to cause coagulation; at the higher temperature the curd may be firmed up more than at the lower temperature, due to the combined effect of the acid and the heat, and the firm curd would more definitely show breaks due to the rising of gas bubbles than would the soft curd. Another possible explanation is a difference in the products of bacterial growth at the two temperatures. This involves whether or not the sweet curdling and the digestion are due to the same enzyme. The results obtained give no definite evidence on this point; it is just as probable that one enzyme might show a different effect at two temperatures as that two enzymes would be produced in different proportions.

<sup>4</sup> Agr. Expt. Sta., Iowa State Col. Res. Bul. 38, 1917.

## SUMMARY

Comparisons were made of the changes produced in milk at room temperature and at 37°C. by various organisms. With certain species the temperature seemed to influence only the rate of change, while with others it also influenced the type. The changes produced at the two temperatures showed the greatest difference with certain of the proteolytic types, although other organisms also showed some differences; with these proteolytic organisms coagulation was much more conspicuous at 37°C. than at room temperature. The type of change was not influenced by the temperature with the true lactic acid organisms, although with certain of them the rate was greatly influenced.

The results suggest the necessity of giving definite information as to the temperature used when the changes produced in milk are recorded in the descriptions of organisms.

## ABSTRACTS OF SOME PAPERS PRESENTED AT THE ANNUAL MEETING<sup>1</sup>

*The Hydrogen Ion Concentration of Cold Storage Butters.* E. H. PARFITT, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

The butters entered in the Butter Storage Contest at the National Dairy Show at Detroit in 1926 were tested to determine the hydrogen ion concentration when entering storage and when leaving storage. The storage period was three months. Butters increased in hydrogen concentration from average pH 6.13 to 6.43. As the score decreased in storage, the pH decreased excepting butters scoring over 94 and under 89. The butters which showed large numbers of proteolytic organisms increased in pH more than the butters low in proteolytic types. Butters with an alkaline flavor were acid, but increased more in pH than did butters not having an alkaline flavor. Butters containing starters varied the least in pH. The pH was determined colorimetrically.

*Methods of Adding Sugar to the Ice Cream Mix.*<sup>2</sup> W. H. MARTIN, Dairy Department, Kansas State Agricultural College, Manhattan, Kansas.

The purpose of this study was to compare, the common practice of adding sugar to the ice cream mix before homogenization, with another method of adding sugar after homogenization. Forty-four mixes were prepared and processed. Viscosity determinations were made, microphotographs of the fat dispersion taken, the rate of freezing and amount of overrun recorded. The body and texture of the ice cream was judged by different individuals

<sup>1</sup> The following abstracts are from papers read at the June meeting of the American Dairy Science Association held at East Lansing, Michigan, June 22 and 23, 1927.—EDITOR.

<sup>2</sup> From a thesis—Sugar in Ice Cream, submitted by Kenneth Miller Renner in partial fulfillment of the requirements for the degree of Master of Science, Kansas State Agricultural College, 1927.

and by allowing the sample to be exposed to room temperature and the rate of melting observed.

The addition of sugar to the ice cream mixes before homogenization increased the viscosity to a greater extent than did the addition of the sugar after homogenization. The fat, as indicated by the micro-photographs, was more highly dispersed in the mixes homogenized without the sugar than in the case of the mix homogenized with the sugar present. Sugar added to the mix after homogenization increased the ease of obtaining overrun. The resistance of the ice cream to melting at room temperature was decreased by the addition of sugar to the mix after homogenization. The method of adding the sugar had practically no effect on the body and texture of the finished ice cream.

*Recent Results Concerning Vitamin B Requirements for Calves.*

S. I. BECHDEL, H. E. HONEYWELL, R. A. DUTCHER AND M. H. KNUTSEN, Pennsylvania State College, State College, Pennsylvania.

Investigations on the vitamin B requirement of calves were started at the University of Minnesota in 1922. Three years ago this investigation was transferred to the Pennsylvania State College where it is still in progress.

The results of the work up to April, 1926, as published in the September, 1926, issue of the JOURNAL OF DAIRY SCIENCE, gave definite evidence that calves, unlike any other species of animal yet studied, will grow normally to maturity and produce normal offspring, on a ration that carries an insufficient amount of vitamin B to support growth and well-being in rats. In this report we stated that the deportment of the experimental calves could be explained only on the basis of vitamin B synthesis by bacteria and other microorganisms in the digestive tract.

The milk from three of the experimental heifers that were fed continuously on the deficient ration for over two years was tested and found to carry as much vitamin B as milk from cows receiving a good winter ration.

In order to make a study of the possible synthesis of vitamin B in the digestive tract, a permanent opening or fistula was made



into the rumen, through the left side of one of the experimental heifers to facilitate the removal of samples of fermented feed for study. Alcoholic extracts, made from the fermented rumen contents have given very positive evidence of the presence of the vitamin B growth factor.

A study of the bacterial flora revealed the presence of an organism which was predominating to the extent of about 90 per cent. Classification studies to date indicate it to belong to the genus *flavo bacterium*. The species does not appear in any classification. This organism was grown in large quantities on the surface of vitamin B free nutrient agar. The bacterial growth was washed from the media with sterile water and the suspension evaporated at a temperature of about 45°C. The dried bacterial cells were fed to young rats as a supplement to a vitamin B free synthetic ration. Results to date have given very positive evidence that the bacterial cells are highly potent in the vitamin B growth factor.

In the course of a few weeks we hope to have our data sufficiently augmented to publish a technical paper concluding that cattle, unlike any species of animal yet studied, have the ability to synthesize their own needed supply of vitamin B through bacterial synthesis in the rumen.

*The Food Value of Milk as Affected by Rations. The Effect of High and Low Protein Rations. (Progress Report.)* W. E. KRAUSS, Ohio Agricultural Experiment Station, Wooster, Ohio.

It was found at the Ohio Agricultural Experiment Station that when cows were fed rations with nutritive ratios of 1:4 and 1:9 production was maintained and the chemical composition of the milk remained unchanged. In order to arrive at the limits of protein feeding rations of wider diversity, nutritive ratios of 1:2 and 1:13 were fed to two groups of three cows each and a study of the nutritive value and chemical composition of the milk produced on these rations begun.

A slightly greater amount of vitamin A, in keeping with the amount furnished in the feed, was found in the milk of the high

protein cows. This difference was not great enough to be of significance in rating the quality of the milk.

An exclusive milk diet of milk from the high protein cows caused the death of rats in a very short time. Some toxic substance is thought to have been a contributing factor in bringing about such early death since the iron content of the milk was found to be practically the same as that of milk, which when fed as the only source of food, enabled rats to live much longer.

When milk from the high protein cows was fed as a supplement to a basal ration no harmful results were observed in rats and calves.

There was some indication that milk from cows fed a ration extremely low in protein is lower in nitrogenous constituents and ash and higher in lactose than that from cows fed a ration extremely high in protein.

*Regarding High and Low Protein Feeding Experiment.* (Progress report.) A. E. PERKINS, Ohio Agricultural College Experiment Station, Wooster, Ohio.

Previous work as reported in Bulletin 389, Ohio Agricultural Experiment Station had shown reasonably satisfactory results with rations ranging from 1:4 to 1:11 in nutritive ratio.

This paper reported the progress to date in the use of rations of still greater extremes, 1:2 and 1:13. This is as far in either direction as it seems possible to go with feeds commonly available on the market, if the rations are kept approximately normal in other respects.

Both extreme rations are undesirable, failing to maintain the cows in proper condition and giving smaller production than the intermediate rations.

A tendency for the cows to go off feed and failure to consume liberally of the rations has featured this phase of the work and may be responsible for the poor condition and production.

There are strong indications that breeding difficulties are developing.

It is felt that the limits of successful high and low protein feeding have been reached.

*The Possibility of Producing Iodized Milk.* C. F. MONROE, Ohio Agricultural Experiment Station, Wooster, Ohio.

If the feeding of iodine to cows leads to the production of iodized milk there must be a large amount of milk sold in Ohio that is relatively rich in iodine. Some of this milk is produced in dairies where iodized milk is desired and the product is specially advertized and sold as "iodized milk." In other dairies the iodized milk is merely incidental to the feeding and administering of iodine to pregnant cows in order to prevent goiter in the new born calf. Many mineral mixtures and proprietary feeds contain added iodine. Therefore, whether or not we are in accord with the therapeutic principle of iodized milk, it is a question that is already with us, that is, if iodine is secreted in the milk from cows receiving iodine.

As yet the present work at the Ohio Station has not advanced to such a stage as to warrant any final conclusions. Samples of milk from various cows which have not received iodine have been examined and we have been unable to find any iodine present. We do not claim this milk to be entirely free from iodine, but we are sure that the iodine content was less than 1 part in 100 million. On the other hand, we have been able to find iodine in the milk of cows which received iodine in the form of potassium iodide, the amount varying between 1 part in 100 million to 1 part in 10 million.

The rate of feeding has been 0.1 gram of potassium iodide daily, or approximately 0.08 gram of iodine. At first the potassium iodide was mixed with the salt and this feed mixed with grain. Later we found it more convenient to make a solution of potassium iodide of such a strength that 25 cc. of solution contains 0.1 gram of potassium iodide. This amount constitutes the daily dose, and is sprinkled over the dry grain in the evening.

*The Adaptation of the MacDonald Process for the Removal of Onion Flavor and Odor in Milk to Creamery Practice.* C. ELMER WYLIE, University of Tennessee.

The MacDonald process for removing onion flavor and odor from milk was discovered by Dr. Margaret B. MacDonald, of the

Tennessee Agricultural Experiment Station.<sup>3,4</sup> Briefly, the process, as determined with small quantities of milk, consists in the mixing of mineral oil with onion milk, which absorbs the onion flavor (allyl sulfide); the separation of the oil from the milk by gravity and filtration; washing the oil; and driving the onion flavor off and sterilizing the oil by heat or live steam. The oil is then ready for use again.

The purpose of this paper is to show the preliminary adaptation of this process to creamery practice and equipment.

A 40-gallon pasteurizing tank of the Sharples Emulsifying unit was used for the experiments at the University of Tennessee Creamery. Seven gallons of onion milk was placed in the tank, and five pints of Lilly Colorless Mineral Oil was added. The agitator was started at a *reduced* speed, and the steam turned on in the water of the double jacket in order to heat the milk. The milk was agitated, and was heated to 115°F. It required about 12 minutes to raise the temperature to that point. The agitator was stopped several times to allow the oil to rise to the top and to prevent too much agitation. The milk was left undisturbed for three minutes. After the oil had risen a sample was drawn until no oil appeared in the milk. This sample was poured back into the tank and another sample drawn to taste for onion. There was little if any evidence of onion flavor or odor in the milk. The milk was then drawn off through a cotton pad strainer into a milk can. Under the strainer was placed a special oil saver. This was designed to keep the oil from coming into contact with the cotton filter pad by keeping a layer of milk above the cotton pad. The oil was then drawn out into a can and the tank flushed out with cold water into the same can. The milk was poured back into the tank and the entire process repeated. The milk was then cooled and placed in cold storage. The taste of the milk at this stage showed no onion flavor or odor.

All of the oil was poured into the tank and first washed with cold water to remove the milk, and then with a 10 per cent solution of washing soda. This wash water was drained off, and the

<sup>3</sup> Circular No. 14, Tennessee Experiment Station.

<sup>4</sup> Journal of Home Economics, Vol. 19, No. 2, February, 1927.

oil was washed twice with fresh water in amounts large enough for the agitator to work. Live steam was turned directly into the oil until it reached a temperature of 180°F. This continued until all onion flavor and odor was driven off and the oil sterilized. The oil and water were then cooled in the tank by running cold water into the double jacket. The oil and water were drawn off into a glass bottle equipped for drawing the water off the oil, and the oil was then ready to use again.

The oil which sticks to the vessels was washed out of the tanks and strainer with a 10 per cent solution of strong alkali. Trials were made with thin cream, which gave similar results, the oil and cream being used in equal amounts for each treatment. The following table shows the results of one of the trials with milk.

	Original	Two treatments Treated
1. Milk, pounds.....	59	58.5 recov- ered
2. Milk temperature.....	75°F.	115°F.
3. Oil temperature.....	Room tem- perature	115°F.
4. Lilly Oil, pounds.....	9	8.8 recov- ered
5. Fat, per cent.....	3.4	3.4
6. Bacteria per cubic centimeter.....	114,000	62,000
7. Taste of milk.....	Onion	No onion
8. Milk recovered, per cent.....		99.1
9. Oil recovered, per cent.....		97.7

*A Statistical Study of the Babcock Test.* D. H. NELSON, Division of Dairy Industry, University of California.

Some two thousand tests were made of samples of normal unpreserved milk. The test bottles and pipettes were re-calibrated and no tolerance allowed. All results in which the fat columns were not amber colored, translucent and free from visible suspended particles were discarded. Independent readings were made by members of the Dairy Industry Division who were instructed to estimate readings to the nearest hundredth per cent.

The probable error of reading the test is  $\pm 0.02$  with a possible variation between different readers of 0.10 per cent. Greater

variations were observed when the fat columns were not amber colored, translucent and free from visible suspended particles. The probable error of pipetting the sample is completely overshadowed by the error of reading the test. The probable error of the Babcock Method under favorable conditions is  $\pm 0.02$  which is due mainly to the method of reading. The Babcock Test has a wide variability which is affected by the color and clearness of the fat column as well as the difficulty of reading the menisci.

The fat content of the milk has no appreciable effect on the probable error of pipetting the sample or of the method.

On each of six samples of milk forty-four tests were made by the Babcock Method and sixteen tests were made by the Mojonnier Method. The averages of the two methods on each sample were found to agree within the probable error of the Babcock Method.

*Opportunities for Dairy Economic Research.* ROY C. POTTS, Chairman Committee on Economic Phases of the Dairy Industry, Department of Agriculture, Washington, D. C.

A paper was presented by Mr. Potts on the above subject in which he discussed some of the opportunities and needs for dairy economic research, and presented good reasons for the organization of a Dairy Economic Section of the Association.

The farm value of dairy production amounts to over two and one-quarter billions of dollars annually. The dairy industry is complex by the very nature of its product, which reaches consumers in the form of numerous manufactured products. The dairy industry must be conducted on a sound economic basis if it is to be permanently successful. The marketing and distribution of dairy products must be conducted in accordance with sound business principles and methods. Economists in our agricultural colleges and universities studying the economic phases of the industry, need the close contact that this organization can give them. They need the help of the Departments of Dairy Industry or Dairy Husbandry in our colleges in planning their investigations. A Dairy Economic Section of this Association would afford an opportunity for the dairy economist and the

dairy scientist or technician, to come closer into coöperation with one another in a way that should be helpful to all.

The dairy industry from an economic standpoint is not a fixed and stationary object. Instead it is a rapidly changing, growing, developing business institution which is governed by the wants and desires of nearly one hundred and twenty millions of people. It is one thing today, another tomorrow. Constantly it is undergoing evolutionary change in accordance with economic law.

The economic problems of the dairy industry know no bounds such as are determined and fixed by state lines. Therefore, the study of many of the economic problems of the dairy industry must be as wide as the industry which suggests the need of much coördination of work among research agencies throughout the industry.

If this Association is to serve the dairy industry in a real scientific and practical way as a fact finding and fact disseminating institution, it must coördinate its forces with the forces of other institutions and agencies to the end that it is able to render the largest service to the industry.

I like to think of our economic research in dairying from the standpoint of applied economics and yet that should hardly be necessary, for if our economic research lacks practical application it is not practical economics. At this stage we ought not to waste much time on things that are not practical.

## SPECIAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

A special meeting of the American Dairy Science Association was held at Gayoso Hotel, Memphis, Tennessee, October 18, 1927 under the auspices of the Southern Division. A very fine program of special interest to southern dairy workers was given. Among the speakers were: C. W. Larson, Chief, Bureau of Dairy Industry, Washington, D. C.; Dr. Bradford Knapp, President, Oklahoma Agricultural and Mechanical College; Mr. J. H. McClain, Dr. J. G. McDowell, Mr. J. B. Parker, Mr. T. E. Woodward, Mr. Ernest Kelly, Mr. R. R. Graves, Mr. M. H. Fohrman, and Mr. W. W. Swett, all from the Bureau of Dairy Industry; and J. H. Frandsen, Editor JOURNAL OF DAIRY SCIENCE.

Tuesday evening a splendid banquet was tendered to the members of the students' judging teams, members of the association and friends of the dairy industry. President Morgan of the University of Tennessee gave the principal address. Mr. W. W. Swett, superintendent of the nineteenth annual students' national judging contest announced the awards of the contest in which 32 teams participated. Mr. Wm. White, superintendent of the eleventh annual national contest in judging dairy products announced the awards in that contest in which 14 teams took part.

Much credit for the success of this special meeting is due to the untiring work of President J. S. Moore and Secretary A. C. Baer of the Southern Division.

Regular sessions of the Production Section and the Official Testing Section were also held at this time. Minutes of these meetings follow.

### PRODUCTION SECTION

A meeting of the Production Section of the American Dairy Science Association was called to order by Chairman A. C. Rags-



dale at 4:30 p.m., October 18, 1927, in the Gayoso Hotel at Memphis.

The minutes of the previous meeting were read and approved.

W. W. Swett, chairman of the Committee on Methods of Conducting Students Contests in Judging Dairy Cattle gave a brief report.

Chairman Ragsdale raised the question as to whether the regular meeting of the section should not be held at the summer meeting of the association, which has been declared the annual meeting.

Because many members cannot attend the summer sessions Mr. Dice suggested that it might be possible to alternate the regular meeting of the section between the summer meeting and the National Dairy Show meeting.

Mr. Anthony moved that the regular meeting of the Production Section be held at the annual meeting of the association. Second by Mr. Gifford. Motion was passed.

E. L. Anthony of West Virginia University was elected chairman and C. F. Huffman of Michigan State College was elected secretary for 1928.

Meeting adjourned at 5:00 p.m.

H. W. CAVE, Secretary.

#### OFFICIAL TESTING SECTION

Minutes of meeting of Official Testing Section, of the American Dairy Science Association, Gayoso Hotel, Memphis, Tenn., 8:00 p.m., October 17, 1927.

I. Reading and approval of minutes of last meeting.

II. Chairman appointed following nomination committee: Harris of Wisconsin, Dice of North Dakota, and Borland of Pennsylvania.

III. Report of Breeds Relation Committee by M. H. Campbell of Vermont. Before giving formal report, Mr. Campbell called upon Harris of Wisconsin, who reported for the Holstein-Friesian Association, and gave summary of proposed Holstein-Friesian Herd Improvement Registry. Lynn Copeland reported for the American Jersey Cattle Club. He stated that there seemed to be

little demand among Jersey men for a herd test. In reporting for the Ayrshire Breeders' Association, C. T. Conklin stated that there were nearly 2600 Ayrshires on test at the present time, or nearly double the number on test at any previous time, and that 95 per cent of the herds on test were starting their third year.

The following recommendations, as reported by Campbell, were approved:

a. (1) This committee believes that a more accurate production may be obtained by using 12 supervisions per year without preliminary milkings, than by using 6 or 8 supervisions with preliminary milkings. We therefore recommend that 12 supervisions without preliminary milkings be used for herd test or herd improvement production records.

(2) This committee believes that the securing of feed records for the benefit of the owner should not be overlooked in connection with herd improvement work. We, therefore, recommend that the owner shall coöperate with the supervisor in furnishing monthly records of feed consumed by each cow, and such records shall be entered in the owner's record book.

(3) We recommend that the maximum milkings be 40 per day, unless supervisor is authorized by the state superintendent to take a larger number. Two cows may be milked at the same time, provided they are near enough for the supervisor to observe both milkers at one time. The supervisor shall run single tests on each sample.

(4) This committee recommends that records made in connection with cow testing (or herd improvement) associations, in compliance with the rules as herein stated, may be accepted for entry in the herd test registry, provided the reports are O.K.'d by the superintendent within the state.

(5) We recommend that daily milk records for each cow under test be kept by the owner. Such records are to be available at all times for inspection by the supervisor, and shall be furnished to the superintendent upon his request. The supervisor should copy on his monthly reports, milk weights for three days preceding the test.

(6) We recommend that a committee be appointed from the

A. R. sections for the purpose of preparing a uniform blank to be used in reporting herd improvement tests.

(7) We recommend that these rules concerning the supervision of herd improvement tests should be sent to each breed association before the next meeting of their board of directors.

After foregoing recommendations were adopted, Campbell continued report as follows:

b. The Breeds Relations Committee of the American Dairy Science Association held a meeting October 16, 1927. Those present were Professors Harris, Reed, Borland, Dice, Williams, and Campbell. Breed Association representatives present were C. T. Conklin and C. M. Cummings, while Professor Harris represented the Holstein Association.

A brief report was made regarding uniform test report blanks. A new supply of 500,000 blanks and 50,000 cards have just been printed. A sufficient supply was sent to each superintendent to last one year. The remainder were divided among the breed associations.

Professor Dice reported on the rules for official testing. Since last year, the Holstein-Friesian Association has printed a new hand book in which are included the rules adopted by this organization. The following recommendations regarding rules were adopted by the committee:

(1) The committee believes that the rule adopted by the A. G. C. C. on the continuation of the one day test in case of a lost milking, is a good thing, and that this suggestion be made to the other breed associations.

(2) The committee recommends that we approve and authorize the A. G. C. C. to print in their rules, the rule asking a breeder to report to the superintendent of official testing in the state, and to the breed association, when a cow is dropped from test.

(3) In view of the fact that the American Milk Goat Association has accepted our uniform rules for the supervision of official tests, except that a supervisor may test 20 goats daily, this committee recommends that the A. R. section of the A. D. S. A. approve the supervision of official tests on milk goats by the superintendents of official tests for the several states.

(4) The American Dairy Science Association has been criticized to some extent for making uniform rules, but not having them enforced in all states. It must be remembered, however, that this Association has no jurisdiction over the various superintendents of official testing.

(5) This committee wishes, however, to recommend that the secretary of the committee send a letter to each breed association, asking which states are not following out the rules, and another to the state superintendents of official testing, with a copy of the uniform rules, and asking if they are following out the rules of the A. D. S. A. The letter to the breed associations might suggest that the field men help gather information regarding rules used in testing. A second letter should be sent to the Dean or Director of the station where rules are not being followed, pointing out the position of the A. D. S. A. in connection with the testing, and making an effort to have these rules adopted in the state.

(6) A suggestion was made that there was no system of uniform blanks to be used by supervisors in various states in taking the barn records, thus causing an inconvenience in filing where check testing was done. It was therefore recommended that a committee be delegated to study the matter of forms for barn records and the possibility of adopting uniform blanks. This committee should report at the next meeting.

IV. Recommendation passed that suggestion be made to Breed Associations, relative to coöperation in the matter of adopting name for the so called Herd Test or Herd Improvement Plan, now known as the Herd Test Plan by the Ayrshire Breeders' Association, and as the Proposed Herd Improvement Plan by the Holstein-Friesian Association.

V. A. C. Ragsdale of Missouri was elected chairman and G. E. Taylor of Michigan as secretary for 1928.

J. M. FULLER, Secretary.

## ANNOUNCEMENT

With the completion of this issue of the JOURNAL OF DAIRY SCIENCE I am glad to turn over the editorial responsibilities to the newly elected editor, Mr. A. C. Dahlberg of Geneva, New York. My wish is that he may have the same whole-hearted support that the American Dairy Science Association has given me during the ten years in which I have had charge of editorial work.

I wish especially to express my appreciation to members of the Editorial Board, both past and present, for their careful and conscientious work in reviewing manuscripts referred to them for criticism. Indeed, much of the success of the JOURNAL is due to the helpful coöperation of members of the Editorial Board.

To the publishers, The Williams & Wilkins Company, who have worked in and out of season to make the JOURNAL mechanically perfect, and who have never failed to meet and adjust the many problems that confront editor and publisher alike, I wish to acknowledge sincere appreciation.

J. H. FRANDSEN.

## DAIRY NOTES

Officers of the American Dairy Science Association elected by mail ballot for 1928-1929 are:

President: G. C. White, Storrs, Connecticut.

Vice-President: A. C. Baer, Stillwater, Oklahoma.

Secretary-Treasurer: J. M. Sherman, Ithaca, New York.

Editor: A. C. Dahlberg, Geneva, New York,

The members of the American Dairy Science Association by majority vote have asked the executive committee to create a new section of the association to be known as the Dairy Economic Section. It is expected that members interested in such a section will soon complete the organization and elect officers.

The Executive Committee has decided upon Madison, Wisconsin, as the place for the 1928 summer meeting of the American Dairy Science Association.

The sixth annual meeting of the Eastern Division of the American Dairy Science Association was held at the Clinton Hotel, Springfield, Massachusetts, September 19 and 20 in conjunction with the Eastern States Exposition. The colleges and experiment stations in this division were all well represented at the meeting and on the program. At the annual banquet C. L. Burlingham, Publisher, *The Breeders Gazette*, Chicago, gave the principal address.

J. G. Watson, livestock editor of the New England Homestead made the announcements and presented the awards in the students' livestock judging contest and Wm. White of the Bureau of Dairy Industry, Washington, D. C., made the announcements and awards in the dairy products judging contest.

Mr. L. H. Hempleman, Department of Dairying, University of Idaho, Moscow, Idaho, has applied for a patent on an invention known as "A Mechanical Device for the Rapid Calculation of Balanced Rations." The inventor claims that this device will greatly simplify the matter of computing rations.



## CALF MEAL STUDIES

### I. LABORATORY EXPERIMENTS IN THE IMPROVEMENT OF PHYSICAL CONDITION

### II. FEEDING EXPERIMENTS WITH COOKED AND UNCOOKED MEAL\*

J. G. ARCHIBALD

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#### INTRODUCTION

For several years the Massachusetts Agricultural Experiment Station has been studying the problem of rearing calves with a minimum of milk. Several experimental calf meals have been worked with, some of which have been fairly satisfactory. In the earlier stages of the investigation, the empirical feeding trial was the sole method of attack, but more recently an attempt has been made to improve the meal which produced the most satisfactory growth, keeping in mind the underlying reasons for the excellence of milk as a food. One of these reasons is its physical condition. Milk is one of the best natural examples of an almost perfect emulsion, its solids (fat excepted) being in either colloidal or molecular dispersion, and remaining so indefinitely if bacterial action and other external influences are inhibited. The idea occurred that it might be possible to improve our best calf meal in this respect and, as a search of the literature revealed that very little had ever been done on the problem, it was considered worthy of investigation.

#### METHOD OF PROCEDURE

It was considered that possible improvement in the physical condition of a calf meal, and hence of the gruel made from it, might be effected in three ways: (1) by increase in fineness of division of the solids, (2) by addition of a protective colloid to aid in maintaining the dispersion of the solids, and (3) by partial cooking of the mixture.

\* Published with the approval of the Director of the Massachusetts Agricultural Experiment Station. Received for publication November 10, 1927.



The laboratory method used to judge the efficacy of these several measures was the suspension test, i.e., the ability of the solids of a suspension made from the meal to remain dispersed in the liquid. The suspensions were made by mixing the meal with cold water in the same proportions as have been used in making up the gruels for feeding purposes, which has been  $3\frac{1}{2}$  ounces of meal to one quart of water, about 1 to 10.

The majority of the tests were made in 250 cc. beakers, using 200 cc. of water and 20.97 grams of meal. A few of the tests where the application of heat was not involved were made in tall glass cylinders, using a column of liquid 11 inches in height.

Combinations of all three measures were worked with in the laboratory and where a considerable improvement that could be effected in practice was noted, it was subjected to the following tests: (1) Feeding trials with calves, growth being noted by the increase in live weight, height at withers, and heart girth, during each week, and (2) digestion experiments of a week's duration with some of the calves.

#### I. LABORATORY EXPERIMENTS

The calf meal used for the work was designated as meal number 7. In earlier feeding trials it had produced growth in calves during the period from three weeks to four months of age, at the average rate of 1.2 pounds daily, 360 pounds of dry matter being required for 100 pounds of gain, 250 pounds of the dry matter coming from the calf meal, the balance from small amounts of whole and skim milk and what rowen the calves would eat. The meal was formulated from the following ingredients:

	<i>pounds</i>
Soluble blood flour.....	5
Skim milk powder.....	15
Linseed meal.....	15
Red dog flour.....	15
Low-grade oat meal.....	30
Corn starch.....	19
Calcium chloride.....	$\frac{1}{2}$
Sodium chloride.....	$\frac{1}{2}$
Total.....	100

The meal used for laboratory tests was prepared in the same manner as that used in the actual feeding experiments, the ingredients being thoroughly mixed and the mixture sifted through a 2 mm. sieve. The siftings were ground in a laboratory mill until they would pass the 2 mm. sieve, and were then mixed with the rest of the meal.

In some of the laboratory tests a good grade commercial calf meal was used in comparison with the number 7 meal. Judging by the manufacturer's statement it was similar in make-up to

TABLE 1

	NUMBER 7 MEAL (AVERAGE OF FOUR ANALYSES)	COMMERCIAL MEAL (ONE ANALYSIS)
	per cent	per cent
Moisture as fed.....	8.5	8.7
<i>Dry matter:</i>		
Crude protein.....	24.6	17.7
Ether extract.....	4.0	4.0
N-free extract.....	64.4	69.2
Crude fiber.....	2.7	4.6
Ash.....	4.3	4.5
	NUMBER 7 MEAL	COMMERCIAL MEAL
	per cent	per cent
Larger than 2 mm.....	0.23	1.38
Larger than 1 mm.....	13.12	14.51
Larger than $\frac{1}{2}$ mm.....	22.46	33.93
Larger than 100 mesh.....	41.95	55.61
Smaller than 100 mesh.....	58.05	44.39

the experimental meal. The proximate chemical analyses and the mechanical analyses of the two meals are given in table 1.

The experimental meal was somewhat superior theoretically to the commercial article. It was in somewhat finer mechanical condition and contained considerably more protein and less fiber. Microscopic examination of its finer material revealed a maximum particle size of about 50  $\mu$ , with most of the particles ranging from 2 to 3  $\mu$ , as compared with a maximum size of 100 $\mu$  or more, in the finer material from the commercial meal, and much greater variation in size of particles.

The results of a suspension test with the two meals are given in table 2. The tests were made in 250 cc. beakers.

This adds further evidence in favor of the superior physical condition of the experimental calf meal. Similar tests were made

TABLE 2  
*Suspension test of calf meals*

ELAPSED TIME AFTER MIXING	AMOUNT OF SEDIMENT		CHARACTER OF THE SUPERNATANT LIQUID	
	No. 7 meal	Commercial meal	No. 7 meal	Commercial meal
	inches	inches		
5 minutes	$\frac{1}{4}$	$1\frac{1}{2}$	Turbid	Clearing at the top
15 minutes	$\frac{1}{4}$	$1\frac{1}{8}$	Still turbid	Continues to clear
1 hour	$\frac{1}{8}$	$1\frac{1}{4}$	Still turbid	Gradually clearing
18 hours	$\frac{1}{4}$	$1\frac{1}{4}$	Still turbid	Upper portion fairly clear

*Note.* The decreasing values for amount of sediment in the later readings are due to more complete settling of the sediment.

TABLE 3  
*Suspension test of calf meal No. 7 in comparison with skim milk powder*

ELAPSED TIME AFTER MIXING	AMOUNT OF SEDIMENT			CHARACTER OF SUPERNATANT LIQUID		
	Skim milk powder (spray dried)	Skim milk powder (drum dried)	No. 7 calf meal	Skim milk powder (spray dried)	Skim milk powder (drum dried)	No. 7 calf meal
		inches	inches			
15 minutes	None	$1\frac{1}{4}$	$\frac{1}{2}$	Does not clear	Clearing	Quite turbid
30 minutes	None	$1\frac{1}{4}$	$\frac{1}{4}$	at all, solids	Clearing	Clearing slightly
1 hour	None	$1\frac{1}{8}$	$\frac{1}{8}$	remain in	Clearing	Not much change
24 hours	None	$1\frac{1}{4}$	$\frac{1}{4}$	perfect sus- pension	Quite clear	Translucent but not clear

on it in comparison with skim milk powder. These were made in tall glass cylinders. The results are reported in table 3.

The number 7 meal occupied a position about midway between the two types of skim milk powder insofar as the suspension test is concerned. The ideal meal in this respect would be one that would remain in as perfect dispersion as did the "spray dried"

skim milk powder. In the nature of things this is a practical impossibility but what improvement could be made was attempted by the methods outlined on p. 119.

### 1. Increase in fineness of division of the solids

This was effected by grinding the meal in a ball mill until it would all pass through a sieve having 100 meshes to the linear inch. The effect of this treatment on degree of dispersion is shown by the suspension test reported in table 4. The data on the original meal are a repetition, for comparison, of those in table 2.

TABLE 4  
*Effect of fine grinding on degree of dispersion of the No. 7 meal*

ELAPSED TIME AFTER MIXING	AMOUNT OF SEDIMENT		CHARACTER OF THE SUPERNATANT LIQUID	
	No. 7 meal (original)	No. 7 meal (100 mesh)	No. 7 meal (original)	No. 7 meal (100 mesh)
	<i>inches</i>	<i>inches</i>		
5 minutes	$\frac{1}{2}$	Boundary between liquid and sedi- ment not distinct	Turbid	Very turbid
15 minutes	$\frac{1}{2}$	$1\frac{1}{2}$	Still turbid	Very turbid
1 hour	$1\frac{1}{2}$	$1\frac{5}{8}$	Still turbid	Very turbid
18 hours	$\frac{1}{2}$	$1\frac{5}{8}$	Still turbid	Quite turbid, muddy brown color

The grinding increased somewhat the ability of the meal to remain in suspension, but reducing the meal to such a degree of fineness was so time-consuming as to be impracticable; also, this fine meal had a decided tendency to "ball" or "lump" when wetted, and was much harder to mix with water than was the original meal. The apparent larger amount of sediment in the 100 mesh product is due to the much bulkier nature of the suspended solids, the particles being so fine that they did not settle out into a compact layer of sediment, such as was noted with the original meal.

### 2. Addition of a protective colloid

Gelatin was used in this phase of the work, the percentage strengths employed being  $\frac{1}{10}$ ,  $\frac{1}{2}$ , 1, 2 and 5 per cent of the weight of the dry meal. The gelatin was dissolved in the water used for making the gruel before adding the meal. Table 5 shows the effect of gelatin when added to a suspension of the original meal made with water at room temperature.

TABLE 5  
*Effect of gelatin on degree of dispersion (cold water suspension)*  
Percentage strength of gelatin

ELAPSED TIME AFTER MIXING	NONE	0.1 PER CENT	0.5 PER CENT	1 PER CENT	2 PER CENT	5 PER CENT
Sediment						
	<i>inches</i>	<i>inches</i>	<i>inches</i>	<i>inches</i>	<i>inches</i>	<i>inches</i>
5 minutes	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$
15 minutes	$\frac{1}{8}$	1	$\frac{15}{16}$	$\frac{9}{16}$	$\frac{9}{16}$	$\frac{1}{8}$
1 hour	$\frac{1}{8}$	$1\frac{1}{8}$	$\frac{15}{16}$	$\frac{15}{16}$	$1\frac{1}{8}$	$\frac{15}{16}$
24 hours	$1\frac{1}{8}$	$1\frac{1}{8}$	$1\frac{1}{8}$	1	$1\frac{1}{8}$	$\frac{15}{16}$
Supernatant liquid						
5 minutes	Very turbid	Very turbid	Very turbid	Very turbid	Very turbid	Very turbid
15 minutes	Very turbid	Very turbid	Very turbid	Very turbid	Very turbid	Very turbid
1 hour	Very turbid	Very turbid	Very turbid	Very turbid	Very turbid	Very turbid
24 hours	All tests disturbed by fermentation					

Apparently gelatin had little or no effect on the degree of dispersion of a cold water suspension of the original meal. Its effect when the suspension was heated, and on the 100 mesh meal is discussed on pp. 127, 128 and 129.

### 3. Partial cooking of the mixture

The suspensions were made up as before and gentle heat was applied with constant stirring until a definite temperature was reached. The temperatures chosen were 37°C. (blood heat) and 60°C. (the coagulation temperature of protein). With the 37°

tests the gruels were held at that temperature for several minutes but the 60° tests were brought to that temperature and removed from the source of heat at once. The temperature in every case was raised quite slowly in order that the full effect of the heat might be obtained. The Number 7 meal and the commercial

TABLE 6  
*Effect of heating on suspensions of calf meal*

ELAPSED TIME AFTER MIXING	AMOUNT OF SEDIMENT		CHARACTER OF THE SUPERNATANT LIQUID	
	No. 7 meal	Commercial meal	No. 7 meal	Commercial meal
Temperature—20°C. (room)				
	<i>inches</i>	<i>inches</i>		
5 minutes	$\frac{7}{8}$	$1\frac{1}{8}$	Turbid	Clearing at top
15 minutes	$\frac{7}{8}$	$1\frac{1}{8}$	Still turbid	Continues to clear
1 hour	$1\frac{1}{8}$	$1\frac{1}{8}$	Still turbid	Gradually clearing
18 hours	$\frac{7}{8}$	$1\frac{1}{8}$	Still turbid	Upper portion fairly clear
Temperature—37°C.				
5 minutes	$1\frac{1}{8}$	2	Turbid	Clearing at top
15 minutes	$1\frac{1}{8}$	$1\frac{1}{8}$	Does not clear	Clearing slightly
1 hour	$1\frac{1}{8}$	$1\frac{1}{8}$	Does not clear	No change
18 hours	$1\frac{1}{8}$	$1\frac{1}{8}$	Does not clear; very turbid	No change
Temperature—60°C.				
5 minutes	$2\frac{1}{8}$ *	$1\frac{1}{8}$	No supernatant liquid	Quite turbid
15 minutes	$2\frac{1}{8}$	$1\frac{1}{8}$	Very slight layer of supernatant liquid	Still turbid
1 hour	$2\frac{1}{8}$	$1\frac{1}{8}$	Layer slightly deeper	Still turbid
18 hours	$1\frac{1}{8}$	$1\frac{1}{8}$	Layer $\frac{1}{2}$ inch in depth	No change

\* Entire column—not settled out any.

meal were both used in this series of tests. The results are tabulated in table 6, the data in table 2 being repeated for ready comparison.

Heating the mixtures increased the ability of the meals to remain in suspension, noticeably at 37°, markedly at 60°. When subjected to the latter temperature the solids of the gruel re-

maintained in practically complete suspension for an hour and had not settled out to any great degree at the end of eighteen hours. The effect was somewhat more marked with the Number 7 meal than with the commercial meal.

TABLE 7  
*Effect of a combination of fine grinding and heating*

ELAPSED TIME AFTER MIXING	AMOUNT OF SEDIMENT	CHARACTER OF SUPERNATANT LIQUID
	No. 7 meal (100 mesh)	No. 7 meal (100 mesh)
Temperature—20°C. (room)		
	<i>inches</i>	
5 minutes	Boundary between liquid and sediment not distinct	Very turbid
15 minutes	1½	Very turbid
1 hour	1½	Very turbid
18 hours	1½	Quite turbid, muddy brown color
Temperature—37°C.		
5 minutes	Not definite, about 2 inches	Very turbid, slightly darker than the unheated
15 minutes	Not definite, about 2 inches	Very turbid, slightly darker than the unheated
1 hour	1½	Quite turbid
18 hours	1½	Quite turbid
Temperature—60°C.		
5 minutes	None—uniform throughout	None—uniform throughout
15 minutes	2½	Slight layer at top
1 hour	2½	One-quarter inch layer at top, fairly turbid
18 hours	1½	Quite turbid

#### *4. Partial cooking and fine grinding*

Portions of the 100 mesh meal were mixed with water and heated as described in the previous section. The data on the suspension tests appear in table 7, a portion of the data in table 4 being repeated for comparison. The Number 7 meal only was used in these tests.

The effect of heat on the finely ground meal was similar to that

on the meal as ordinarily fed. The efficacy of fine grinding, as set forth in table 4, for the unheated suspension, was not so noticeable when 37° heat was applied and was completely offset by heating

TABLE 8  
*Effect of adding gelatin and heating*  
Percentage strength of gelatin

ELAPSED TIME AFTER MIXING	NONE	$\frac{1}{16}$ PER CENT	$\frac{1}{8}$ PER CENT	1 PER CENT	2 PER CENT	5 PER CENT
Temperature—37°C.						
	<i>inches</i>	<i>inches</i>	<i>inches</i>	<i>inches</i>	<i>inches</i>	<i>inches</i>
<i>Sediment</i>						
5 minutes	$\frac{7}{8}$	1	$1\frac{1}{8}$	$1\frac{1}{8}$	$1\frac{1}{8}$	$\frac{7}{8}$
15 minutes	$1\frac{1}{8}$	$1\frac{1}{8}$	$1\frac{1}{8}$	$1\frac{1}{8}$	$1\frac{1}{8}$	$1\frac{1}{8}$
1 hour	$1\frac{1}{8}$	$1\frac{1}{8}$	$1\frac{1}{8}$	$1\frac{1}{8}$	$1\frac{1}{8}$	1
24 hours	All tests disturbed by fermentation					
<i>Supernatant liquid</i>						
5 minutes	Very turbid	Very turbid	Very turbid	Very turbid	Very turbid	Very turbid
15 minutes	Very turbid	Very turbid	Very turbid	Very turbid	Very turbid	Very turbid
1 hour	Clearing some	Still very turbid	Still very turbid	Still very turbid	Still very turbid	Still very turbid
24 hours	All tests disturbed by fermentation					
Temperature—60°C.						
<i>Sediment</i>						
5 minutes	$2\frac{1}{2}$	$2\frac{1}{2}$	$2\frac{1}{2}$	$2\frac{7}{8}$	$2\frac{1}{2}$	$2\frac{7}{8}$
15 minutes	$2\frac{1}{2}$	$2\frac{7}{8}$	$2\frac{7}{8}$	$2\frac{7}{8}$	$2\frac{1}{2}$	$2\frac{7}{8}$
1 hour	$1\frac{1}{8}$	$1\frac{1}{8}$	$1\frac{1}{8}$	$1\frac{1}{8}$	$1\frac{1}{8}$	$1\frac{1}{8}$
24 hours	All tests disturbed by fermentation					
<i>Supernatant liquid</i>						
5 minutes	Little or none—solids distributed throughout almost the entire column of liquid					
15 minutes						
1 hour	Similar in all, the cooking produced a gel which slowly settled out, leaving the supernatant liquid quite clear					
24 hours	All tests disturbed by fermentation					

to 60°C., (see table 6). Presumably the cooking partially hydrolyzed the starches and gums in the meal so that a hydrogel was formed which was viscous enough to hold in suspension not only



the fine particles of the 100 mesh meal but also the coarser particles of the original meal.

### 5. *Partial cooking and addition of gelatin*

The strengths of gelatin used were those already noted in section 2, and the method of preparing and heating the suspensions was as previously described. The Number 7 meal was used for all the tests, results appearing in table 8 (compare with table 5).

TABLE 9  
*Effect of fine grinding and adding gelatin*

ELAPSED TIME AFTER MIXING	AMOUNT OF SEDIMENT		CHARACTER OF SUPERNATANT LIQUID	
	Control	5 per cent gelatin	Control	5 per cent gelatin
	<i>inches</i>	<i>inches</i>		
5 minutes	Settling evident but no definite sediment	No definite sediment	Very slight layer at top, not clear	None
15 minutes	Settling slowly but no definite sediment	Settling some but not as much as the control	The slight layer is still very turbid	Very slight layer—very turbid
1 hour	1½	No definite sediment	Still quite turbid	Very turbid
2 hours	1½	No apparent change from one hour	Translucent rather than turbid	Very turbid
18 hours	1	Very little change, holding up well	Fairly clear	Very turbid

With 37° heat the gelatin helped a little during the first hour or so. The 5 per cent test held the solids in suspension noticeably better than did any of the others, while the control test was somewhat inferior to any of the gelatin tests. With 60° heat none of the gelatin tests were in any way superior to the control. Presumably heating to this temperature hydrolyzed the starch so that it had a protective action equal to, if not greater than, the added gelatin.

### 6. *Fine grinding and addition of gelatin*

For this test the 5 per cent strength of gelatin only, was used, in a suspension of the 100 mesh meal. Results are given in table 9.

The addition of the gelatin increased considerably the ability of the 100 mesh meal to remain in suspension. The result here is somewhat different from that obtained when gelatin was added to a suspension of the original meal (see table 5). The assumption is that in the latter case the particles were not fine enough for the protective action of the colloid to have any appreciable effect.

TABLE 10  
*Effect of a combination of all three agencies*

ELAPSED TIME AFTER MIXING	AMOUNT OF SEDIMENT (INCHES)		CHARACTER OF SUPERNATANT LIQUID	
	Control	5 per cent gelatin	Control	5 per cent gelatin
5 minutes	Uniform dispersion throughout in both			
15 minutes	Slight layer of clear supernatant liquid at top of each column, otherwise uniformly dispersed			
1 hour	The slight layer of clear supernatant liquid in both tests increased slightly during each time interval, but the solids remained uniformly dispersed, with no separation into layers of coarser and finer particles			
2 hours				
18 hours				

### 7. *Fine grinding plus gelatin plus partial cooking*

The tests were identical with those in the last section, except that they were heated to 60°C. The results appear in table 10.

The good effect of the added gelatin noted in the last section was completely offset by heating to 60°C., the gelatin test being no better than the control. The explanation offered is the same as given in section 5 on p. 128.

### CONCLUSION FROM LABORATORY WORK

1. A reasonable degree of fineness is requisite in a calf meal, but very fine grinding is impracticable, and may be unnecessary.

2. Gelatin, taken as a type of protective colloids, is without effect on calf meal gruels as ordinarily prepared. This is con-

sidered to be due to the fact that the particles are too large for the protective action to take place.

3. Moderate heating (not above 60°C., because of protein coagulation above that temperature) seems to be the most efficacious manner of improving the physical condition of calf meal gruels as manifested by increasing the dispersion of the solids. It accomplishes the same end as does fine grinding, or addition of gelatin, or both, and is much more nearly practicable than the first mentioned.

## II. FEEDING EXPERIMENTS

Having reached the conclusion just stated, the next step was to carry on some feeding experiments to ascertain whether the partially cooked meal was an improvement in practice over the raw meal.

Ten grade Holstein bull calves ranging in age from one to four days were obtained and reared on whole milk, skim milk and calf meal Number 7 in the usual manner.<sup>1</sup> They were divided into two groups of five each, one group receiving the raw meal, the other the meal heated to 60°C. as described on p. 124. Division into groups was made largely on the basis of live weight at the commencement of the experiment, although apparent vigor was

<sup>1</sup> The following excerpt from Massachusetts Agricultural Experiment Station Bulletin No. 223 describes the method of feeding:

"Our method of feeding has been planned so as to have the calves weaned from whole and skim milk as early as possible. The calves are left with their dams only twenty-four hours, at the end of which time they are taught to drink whole milk from a pail. The milk fed to them is always from one of the lowest testing Holstein cows in the herd, and the maximum fed is six quarts daily. When the calf is a week to ten days old the whole milk is gradually replaced by skim milk. If it is a vigorous animal it will be receiving skim milk entirely by the end of the second week. When the calf is from two to three weeks old the skim milk is gradually replaced, a quart at a time, by a gruel made from the meal which it is desired to give a trial. The gruel is made by mixing the meal with water in the proportion of 3½ ounces of meal to each quart of water. It is stirred up with a little cold water first so lumps will not form, and then the correct amount of warm water is added, and the gruel fed at blood heat, never cold or very hot. Skim milk, while it continues to be fed, is mixed with the gruel at feeding time."

also considered. Average figures for weight, height at withers, and heart girth, at one week of age, appear below.

	WEIGHT	HEIGHT	GIRTH
	pounds	inches	inches
Group 1 (raw meal).....	90.6	29.5	31.7
Group 2 (cooked meal).....	92.0	29.3	30.9

The average age at which the calves first received the calf meal gruel was 19.4 days, Group 1 commencing at 19 days and Group 2 at 19.8 days. All calves continued to receive the gruel until they were four months of age at which time the experiment was

TABLE 11  
*Feed consumption and gains*

GROUP	WHOLE MILK	SKIM MILK	CALF MEAL NO. 7		ROWEN	TOTAL DRY MATTER CONSUMED	TOTAL GAIN	AVERAGE DAILY GAIN	DRY MATTER REQUIRED FOR 100 POUNDS OF GAIN
			As gruel	As dry meal					
	quarts	quarts	pounds	pounds	pounds	pounds	pounds	pounds	pounds
1 (raw meal).....	188	76	171	61	116	378	126	1.05	298
2 (cooked meal)....	185	94	157	65	116	373	115	0.95	334

discontinued. Table 11 contains the records of average feed consumption and gains.

In addition the accompanying figures showing the average growth curves give the results at a glance, (see figs. 1 and 2).

All the records of the feeding trials are consistently in favor of the raw meal, although at the start the two groups were about as identical as it is possible to have groups which are composed of living individuals. The partially cooked meal apparently was less palatable than the raw meal as the amount of liquid feed refused was considerably higher where it was fed. This lower feed consumption explains in part the less favorable growth records, but it was evident all through the experiment that this group of calves were not making as good use of what they actually

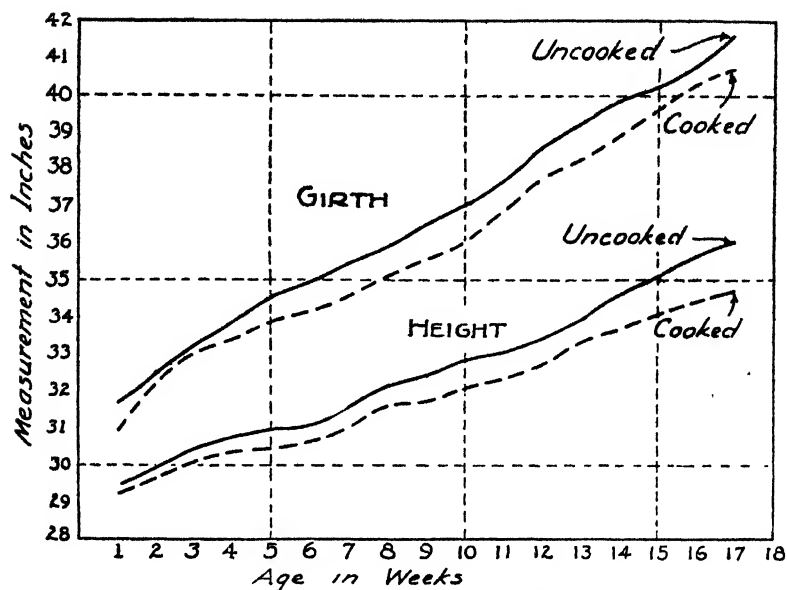


FIG. 1. AVERAGE WEIGHTS IN CALF MEAL EXPERIMENT—5 CALVES IN EACH GROUP

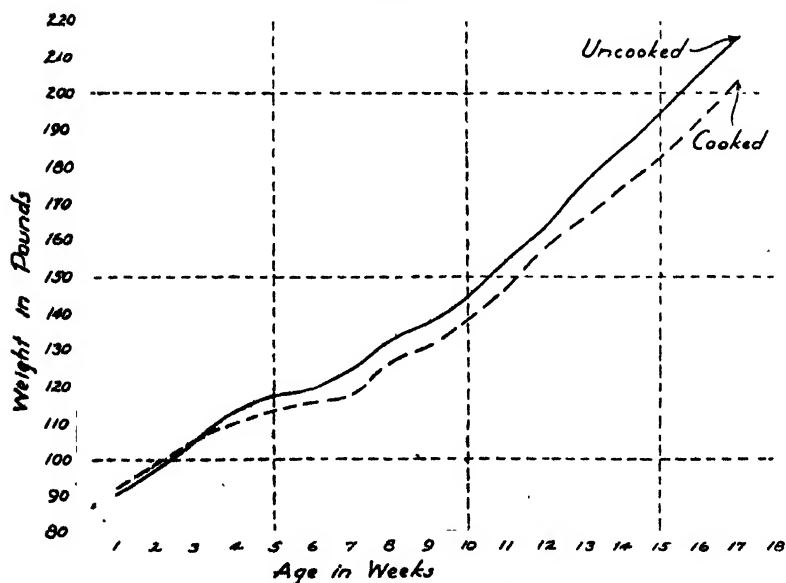


FIG. 2. AVERAGE GROWTH CURVES IN CALF MEAL EXPERIMENT—5 CALVES IN EACH GROUP

consumed as were those receiving raw meal gruel. They showed a greater tendency to scour and to develop the "pot belly" so characteristic of an unthrifty calf. Observation of their behavior leads to the belief that the unsatisfactory results with the cooked meal were largely due to excessive fermentation in the intestinal tract, evidently favored by the partial cooking. If there was any favorable influence due to the improved physical condition of the cooked gruel, it was not in evidence.

Digestion experiments were also carried on with six of the calves. These were conducted in pairs, on calves of the same

TABLE 12  
*Digestibility of calf meal No. 7 (raw and cooked)*

	DRY MATTER	CRUDE PROTEIN	CRUDE FIBER	N-FREE EXTRACT	ETHER EXTRACT
<i>Uncooked meal</i>					
Calf 108.....	72.14	78.81	None	73.54	94.85
Calf 110.....	76.97	79.25	None	76.70	95.24
Calf 112.....	65.81	76.63	None	63.67	95.27
Average.....	71.64	78.23	None	71.30	95.12
	±1.25	±0.61	—	±2.65	±0.09
<i>Cooked meal</i>					
Calf 109.....	73.97	79.45	None	72.84	95.41
Calf 111.....	74.10	73.27	None	74.17	95.04
Calf 115.....	75.71	69.50	None	79.97	93.56
Average.....	74.59	74.07	None	75.66	94.67
	±0.22	±1.94	—	±1.48	±0.38

age, one calf being fed the raw meal gruel and the other the partially cooked gruel. The average age of the calves when the digestion trials were started was nine weeks. Each trial lasted ten days, collection of urine and feces being made on each of the last seven days. The ration fed during the trials was identical in all cases, viz., seven quarts of calf meal gruel containing 24½ ounces of meal, and two quarts of whole milk, per calf daily. In only one instance was any food wasted, the amount of waste being equivalent to 5½ per cent of the total dry matter fed. The summarized data of the digestion experiments appear in table 12.

In total dry matter and nitrogen-free extract the cooked meal was slightly more digestible, while its protein was less digestible. The question may be raised as to why, if the cooked meal was more digestible, it did not induce greater growth than did the uncooked meal. The probability is that the difference was only apparent, being due to bacterial fermentation with the production of large amounts of gases, rather than to true enzymatic digestion. The point to be emphasized is the failure of the cooking to have any marked effect on digestibility, either favorable or otherwise.

TABLE 13  
*Feed consumption and gains on skim milk powder + red dog-hominy*

GROUP	WHOLE MILK	SKIM MILK POWDER	RED DOG-HOMINY	DRY GRAIN* NO. 5	ROWEN	TOTAL DRY MATTER CONSUMED	TOTAL GAIN	AVERAGE DAILY GAIN	DRY MATTER REQUIRED FOR 100 POUNDS OF GAIN
	quarts	pounds	pounds	pounds	pounds	pounds	pounds	pounds	pounds
Raw red dog-hominy.....	167	164	114	93	170	541	198†	1.64	272
Cooked red dog-hominy.....	138	161	129	87	141	511	183	1.50	280

\* This is not the red dog-hominy mixture but a grain mixture composed of corn meal, ground oats, linseed meal, red dog flour and salt.

† At end of four months.

In addition to the results of the feeding trials reported in the previous section, the data from eleven calves used in the study of the general problem of milk substitutes are of significance in this connection. These calves were reared on a gruel made from drum-dried skim milk powder and a grain mixture consisting of equal parts of yellow hominy meal and red dog flour, in the proportion of 3 ounces of skim milk powder and 1½ ounces of red dog-hominy mixture to one quart of water. When the calves became quite hearty (at an age varying from two to three months), the amount of red dog-hominy was gradually increased until they were receiving as much of it as of the skim milk powder, i.e., 3 ounces

per quart. For six of the group the red dog-hominy mixture was cooked to the consistency of a thin porridge with the necessary water and when cooled to about blood heat the skim milk was stirred in, and the gruel fed at once. For the other five, the skim milk powder and red dog-hominy were simply stirred up with lukewarm water at feeding time. Table 13 presents the records of average feed consumption and gains.

The calves fed the raw red dog-hominy were heartier, made somewhat higher gains and required slightly less feed for a given amount of gain than did those fed the cooked gruel.

#### CONCLUSIONS FROM THE FEEDING TRIALS

1. Cooking reasonably fine calf meals, although it changes their physical condition, by increasing the dispersion of the solids, does not appear to exert any favorable effect on growth. Very fine grinding of calf meals for feeding was not undertaken because of its impracticability, hence its effect as compared with cooking has not been measured.

2. Digestibility of a calf meal is not significantly affected by cooking.



# THE ICE CREAM SCORING CONTEST AS A MEANS OF IMPROVING THE QUALITY OF ICE CREAM\*

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## INTRODUCTION

Since 1921 the Dairy Husbandry Department of the Kansas Experiment Station has held seven annual ice cream scoring contests. The quality of the ice cream submitted each year has shown noticeable improvement over the preceding year. The improvement has been especially marked in the sanitary quality as revealed by the bacteriological analyses on the samples submitted. It is not contended that these scoring contests have been solely or even largely responsible for the improved quality of the ice cream in this section of the country, but they certainly have been of educational value to the ice cream makers participating in the contests.

## RULES OF THE CONTEST

The contest is open to any ice cream plant selling any part of its product in the state of Kansas, and several samples from plants in adjoining states have been entered. One or more two-gallon samples may be submitted, but it is expressly stated that they must be from the regular run of the plant and not specially prepared samples. Many plants send in more than one sample, using the contest as a means of securing information on the com-

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† Contribution No. 92 from the Department of Bacteriology, and No. 59 from the Department of Dairy Husbandry.

parative value of certain processes or ingredients. All samples are shipped so as to arrive in Manhattan four days before the contest. This allows time for the scoring of the samples by the official judge, and for making a chemical and bacteriological analysis of each sample.

On the specified date, representatives from the various plants, among whom are ice cream makers, managers, and superintendents, meet in Manhattan for a two day conference. Excellent programs have been given each year by specialists in the theoretical and practical phases of the ice cream industry. A great deal of interest is manifested in these meetings, the educational value of which would be difficult to estimate.

On the second day of the meetings, the samples of ice cream are opened for the inspection of everyone present. The samples are designated by number, and the identity is known only to the owner of the ice cream and the officials of the contest. At this time the official scores are announced, together with the results of the chemical and bacteriological analyses.

No prizes are offered for the high scoring sample, and, in fact, the name of the owner is not even announced. Great care is taken to preserve the educational features of the contest and to eliminate the competitive element. The objective of such a plan is to enable the ice cream makers to profit through the educational aspects of the program and through contacts which the meeting affords, but more especially to see how their product compares with that of others. Many ice cream makers learn through these meetings that a product, which they previously thought to be excellent, is, after all, of mediocre or poor quality. By comparing their own product with others they can see the strong and weak points of each. Improvement, which is the aim of the contest, usually results. That the objective of the contest has been realized is clearly evident when the scores of the ice cream for each successive year are compared.

#### BACTERIOLOGICAL RESULTS

In table 1 are given the lowest, the highest, and the median count of the samples for each separate year, and of the entire

group for the seven years. The median count is obtained by alternately striking out the highest and lowest count until the middle value is reached. This value is believed to be more representative of a group of bacteriological analyses than the arithmetical average, due to the distorting effect of a single high count on the result obtained by averaging.

It will be noted that the median count for the first year of the contest (1921) was 200,000 per gram and that each successive year it was lower than the preceding year. A detailed examination of the individual analyses for each successive year shows very convincingly the improvement in the sanitary quality of the ice cream submitted. In lieu of the mass of detailed figures,

TABLE 1

*The extremes and median counts on ice cream submitted to each of the seven annual scoring contests*

	YEAR							VALUES FOR ALL SAMPLES
	1921	1922	1923	1924	1925	1926	1927	
	28 samples	39 samples	48 samples	53 samples	42 samples	35 samples	46 samples	
Low count.....	5,000	4,000	2,000	3,000	2,000	2,000	3,000	2,000
High count.....	20,000,000	27,000,000	47,000,000	1,000,000	2,400,000	2,500,000	14,300,000	47,000,000
Median count.....	200,000	87,000	80,000	70,000	53,000	46,000	32,000	66,000

table 2 is submitted and presents a fairly clear picture of the improvement in the sanitary quality of the ice cream as evidenced by lower bacterial counts.

Table 2 shows the per cent of the samples containing less than a specified number of bacteria per gram, for each year and for the entire seven years. For illustration, note that in 1921 only 17.9 per cent of the samples contained less than 20,000 bacteria per gram; in 1922 the same percentage obtained, but from 1923 to 1927 there was a consistent increase in the per cent of samples below this value. Again, note that in the first contest only 32.1 per cent of the samples contained less than 50,000 bacteria per gram, whereas in the last contest more than 60 per cent were in this group. A study of this table shows a tendency in each succeed-

ing contest for more samples to be classed in the low count groups, and fewer in the high count groups.

### FLAVOR, BODY AND TEXTURE SCORES

In view of the fact that each year a different judge scored the various samples, it has been difficult to correlate the scores. Some judges regarded an excellent body and texture worth a perfect score, whereas others gave no perfect scores to any of the samples. Another factor that accentuated the difficulty of correlating the

TABLE 2

*The per cent of samples of ice cream in the scoring contests (1921-1927) containing less than specified numbers of bacteria*

	YEAR							VALUE FOR 7 YEARS
	1921	1922	1923	1924	1925	1926	1927	
	28 samples	39 samples	48 samples	53 samples	42 samples	35 samples	46 samples	291 samples
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
10,000 or less.....	14.3	5 1	18.7	15.1	14.3	22.8	17.4	15.5
20,000 or less.....	17.9	17.9	22.9	22.6	35.7	40.0	41.3	28.5
50,000 or less.....	32.1	38.5	37.5	37.7	47.6	51.4	60.9	44.0
100,000 or less.....	35.7	51.3	54.2	58.5	71.4	65 7	67.4	58.8
200,000 or less.....	57.1	59.0	70.8	73.6	78.6	77.1	73.9	70.8
300,000 or less.....	67.9	71.8	75.0	86 7	80.9	80.0	76.1	77.7
500,000 or less.....	78.6	74.3	75.0	90.6	85.7	80 0	80.4	81.1
1,000,000 or less.....	89.3	82.0	81.2	100.0	97.6	85.7	91.3	90.0
Over 1,000,000.....	10.7	18.0	18.8	0	2.4	14.3	8 7	10.0

scores for various years, was the fact that a different score card was used for the last two years. However, the scores each year for the various factors can be grouped into four groups; excellent, good, medium and poor. For example, each judge apparently regarded a certain number of samples as excellent in body and texture, and to these gave a score, which, in his estimation represented the relative value of an excellent sample. With some judges the score allotted to this group of samples was one or two points below perfect, whereas other judges gave perfect scores to all the samples in this group. Similarly, another group of

samples, not good enough to be excellent but better than the average, could be observed in the ratings given by each judge. In this way it has been relatively easy to select from the scores for each year, the number of samples in each of the four groups. This method of grouping the scores has been followed for flavor,

TABLE 3  
*Per cent of samples of ice cream which were classed as excellent, good, medium and poor*

	1921	1922	1923	1924	1925	1926	1927
Flavor score							
Excellent.....	25.0	17.9	18.8	9.6	9.7	17.1	24.4
Good.....	21.5	15.5	27.1	26.9	48.8	51.4	48.9
Medium.....	28.5	43.6	43.8	44.2	29.3	25.7	15.5
Poor.....	25.0	23.0	10.3	19.3	12.2	5.8	11.2
Excellent and good.....	46.5	33.4	45.9	36.5	58.5	68.5	73.3
Medium and poor.....	53.5	66.6	54.1	63.5	41.5	31.5	26.7
Body and texture score							
Excellent.....	32.1	25.6	18.8	17.3	29.3	8.6	15.5
Good.....	28.6	28.2	43.7	51.9	26.8	51.4	48.9
Medium.....	28.6	30.8	31.3	26.9	21.9	28.6	31.2
Poor.....	10.7	15.4	6.2	3.9	22.0	11.4	4.4
Excellent and good.....	60.7	53.8	62.5	69.2	56.1	60.0	64.4
Medium and poor.....	39.3	46.2	37.5	30.8	43.9	40.0	35.6
Total score minus bacteria score							
Excellent.....	3.6	10.2	20.8	11.5	17.1	11.4	15.6
Good.....	21.4	46.2	33.3	32.5	46.3	45.7	73.2
Medium.....	60.7	33.3	37.5	42.3	26.8	34.3	8.9
Poor.....	14.3	10.3	8.4	13.7	9.8	8.6	2.3
Excellent and good.....	25.0	56.4	54.1	44.0	63.4	57.1	88.8
Medium and poor.....	75.0	43.6	45.9	56.0	36.6	42.9	11.2

body and texture, and total score minus the bacteria score. A further grouping of the scores has been made by combining the total number of excellent and good samples for one group, and the medium and poor samples for another group. This method of grouping has been followed for the purpose of determining if there has been any tendency toward an increase in the number of

samples in the better classes of ice cream. Table 3 shows the results of these groupings.

In the first section of table 3 the flavor scores are considered. A study of the figures for the individual groups does not indicate any uniform improvement in the flavor of the samples from year to year. There is, however, a noticeable tendency for fewer samples to fall into the medium and poor groups and a correspondingly greater number to be classed as good and excellent. From 1921 to 1927 there was an increase from 46.5 to 73.3 per cent in the number of excellent and good samples and a corresponding decrease from 53.5 to 26.7 per cent in the number of medium and poor samples.

Improvement in the body and texture of the ice cream, has been less noticeable than the improvement in the flavor. The figures in the second section of table 3 show that the number of samples scoring excellent and good in body and texture increased from 60.7 per cent in 1921 to 64.4 per cent in 1927. Figures for the intervening years show considerable variability in the per cent of high scoring samples, but viewing them as a whole there is some evidence that the body and texture of the ice cream submitted has improved.

Finally, in the last section of table 3 the total scores for all points except bacteria are considered. Here again the improvement over the seven year period is noticeable. The per cent of excellent samples increased from 3.6 to 15.6 per cent, and the per cent of good samples from 21.4 to 73.2 per cent. In 1927 only 2.3 per cent of the samples were classed as poor, as compared with 14.3 per cent in 1921. During the same period the number of samples classed as medium decreased from 60.7 to 8.9 per cent.

#### SUMMARY

1. The sanitary quality of the 291 samples of ice cream entered in seven annual scoring contests held at the Kansas State Agricultural College, has shown a marked improvement from year to year. With a median count of 200,000 bacteria per gram in 1921 there has been, without exception, a consistent reduction each year to 32,000 in 1927.

2. The samples for each year have been grouped into four classes, excellent, good, medium and poor. A study of these groups reveals a definite improvement in the quality of the ice cream from year to year as evidenced by the increase in the per cent of samples in the better (excellent and good) classes.

The per cent of samples classed as having excellent and good body and texture scores increased from 60.7 in 1921 to 64.4 in 1927.

By classifying the samples on a basis of the total score minus the score for bacteria count, the excellent and good samples increased from 25.0 per cent in 1921 to 88.8 per cent in 1927.

3. While it is not contended that these scoring contests have been solely responsible for the improvement in the quality of ice cream as evidenced by these data, they have been a contributing factor in the educational program for the ice cream manufacturers of this section of the country. These scoring contests have been of inestimable value to the ice cream manufacturers of this section. The success of the system in this state and the evident improvement in the quality of the ice cream, commends the adoption of a similar plan by other institutions as a part of their educational program.

# THE KEEPING QUALITIES OF GHEE\*

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## INTRODUCTION

Milk fat is one of the very important human foods. In some parts of the world such as America, Europe and Australia it is used in the form of butter, while in India and certain other countries it is used principally as ghee.

Ghee is prepared by heating unsalted butter on a low fire for a considerable period and then straining; the heating evaporates considerable quantities of the water and the straining removes a part of the curd which the heating has tended to flocculate. The milk of various animals is used in making ghee, but in India the milk of the buffalo is the most widely employed.

The present paper reports certain observations made on the keeping qualities of ghee; these are believed to be of significance in their relationship to the general problem of the keeping qualities of butter.

## USES AND PROPERTIES OF GHEE

Ghee is ordinarily employed on various types of bread and in the cooking of meat, fish, vegetables, rice, etc. Large quantities are used in the sweetmeat industry. It is utilized to a limited extent for medicinal purposes when it is prepared from cows' milk and especially when it is very old.

Because of the temperature used in the making, ghee has a pronounced heated flavor and odor. It never shows the waxy texture of butter and is commonly grainy and brittle. At rather high temperatures there may be considerable oiling off.

Ghee keeps well under temperature conditions that would be considered very unsatisfactory for butter. It seems probable that its manufacture developed because of the unfavorable

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conditions for the storage of butter prevailing in certain countries where the temperatures are often high and the facilities for cooling limited.

#### METHODS

*Preparation of ghee.* The ghee used was prepared by heating unsalted butter made from cows' milk in small quantities in an aluminum utensil over a gas burner. The temperature to which the butter was heated in making what was considered normal ghee varied from 122° to 130°C., depending somewhat on the season; in addition to the temperature, the sound and foaming of the heating material and the appearance of the curd were considered in deciding as to when the heating should be stopped.

*Preparation of butter fat.* The butter fat was prepared by melting the butter, without heating it hotter than 55°C., allowing the water and curd to settle and then decanting the fat and filtering it through filter paper.

*Storage of ghee and other materials.* The normal and abnormal ghee and the butter and butter fat used for comparison were all stored in glass containers of capacities of  $\frac{1}{2}$  or 1 pint. Except in the trials on the influence of light, the samples were stored in the dark; when held in the dark the containers were wrapped in paper as an additional protection.

*Materials used for comparisons.* The materials used for any comparison always came from the same lot of butter.

*Judging the materials.* The judging involved only the detection of defects; no attempt was made to set a definite score.

*Chemical methods.* In securing the samples of the various materials, cores were removed through the entire depths so as to compensate for any separation that may have occurred. Ghee and the purified butter fat were used directly for the fat constants, while the butter and the ghee to which water had been added were melted and the fat decanted and filtered. The samples taken for analysis were put in  $\frac{1}{2}$ -pint milk bottles, warmed in a water bath and thoroughly stirred before weighing out.

Nitrogen determinations were made by the Kjeldahl method.

The moisture determinations were made with a Mojonnier tester following the procedure used with butter. For the acid values (milligrams of KOH required to saturate the free acids in 1 gram of fat) 20 grams (or occasionally only 10 grams) of fat were titrated with  $N/10$  KOH after dissolving the fat in hot neutral alcohol. The iodine number was determined by the Hanus method, the Reichert-Meissl number by the Leffman and Beam method, the melting point by the Wiley method, while for the refractometer readings a Zeiss-Butyro-Refractometer was used.

#### EXPERIMENTAL

##### *Keeping qualities of ghee*

*Keeping qualities of ghee and butter.* A number of comparisons of the keeping qualities of ghee and butter were made at both room temperature and cooler temperature. In each trial the ghee kept very much better than the butter. At room temperature the butter was commonly off in flavor and odor in from one to two weeks while the ghee usually showed little if any deterioration throughout the entire holding periods, which varied from nineteen to twenty-three weeks. In the cooler the butter kept somewhat longer than at room temperature but its keeping qualities were still very limited as compared to those of ghee.

*Keeping qualities of ghee and butter fat.* The keeping qualities of ghee and butter fat were compared in two trials. Both the ghee and the butter fat kept very well at either room temperature or cooler temperature. In one comparison the ghee at both temperatures was off in flavor and odor after about nine weeks while the butter fat was still satisfactory after twenty weeks; the off condition in the ghee involved a cheesy odor which seemed to accompany the growth of mold.

One comparison included a second lot of butter fat which had been heated to  $130^{\circ}\text{C}$ . after its preparation in the usual way. This also kept very well at the two temperatures used during a holding period of twelve weeks.

*Keeping qualities of normal ghee and abnormal ghee.* Abnormal ghee was prepared by adding sterile water to approximate the water content of butter or by omitting the straining so that the

curd content was high. The keeping qualities of such ghee were compared with those of normal ghee.

Two comparisons of ghee and ghee with added water were made; in one only room temperature was employed, while in the other room temperature and cooler temperature were used. The ghee with the added water deteriorated rather quickly in both comparisons while the normal ghee was still satisfactory after the holding periods, which were twelve weeks in one instance and nineteen weeks in the other. The ghee with added water became cheesy and showed a growth of mold. In the comparison involving two holding temperatures there seemed to be little, if any, difference between them.

Normal ghee and unstrained ghee were compared in three trials, two including only room temperature, while the third included both room temperature and cooler temperature. In the trials made at room temperature only, both the normal and unstrained ghee remained satisfactory throughout the holding periods, one of which was seventeen weeks and the other nineteen weeks. In the third trial the unstrained ghee became cheesy after two weeks at room temperature and after three weeks at cooler temperature, while the normal ghee was satisfactory at both temperatures throughout the twenty-three-week holding period; the off condition again seemed related to the development of mold.

*Keeping qualities of ghee in the dark and in the light.* The keeping qualities of ghee in the dark and in diffuse light were compared at room temperature in seven trials. Two of the lots of ghee were made from good butter while five were made from poor butter.

In six of the seven trials the ghee held in the diffuse light developed a tallowy odor in from two to seven weeks, while in the remaining one, which involved ghee made from good butter, there was no tallowiness after the ten weeks holding. None of the samples of ghee held in the dark developed tallowiness. Two of the lots of ghee which showed the tallowy condition were also bleached; these had remained solidified throughout the holding period, while the others showed some liquid fat at the surface.

*Keeping qualities of ghee made from poor butter.* Six lots of ghee were prepared using poor butter and the keeping qualities of these studied. It was very evident that the quality of the butter influenced the quality of the ghee made from it and that the heated flavor and odor developed during the making of the ghee did not cover up serious flavor and odor defects in the original butter. This complicated the study of the influence of holding on the ghee.

In two of the lots of ghee from poor butter there seemed to be a deterioration on holding, while with the others there was no evident change after periods of from sixteen to twenty weeks. The deterioration did not involve a cheesy condition as was the case with the deterioration of normal and abnormal ghee already referred to.

TABLE 1

*Nitrogen and moisture contents of lots of ghee prepared at different temperatures*

GHEE HEATED UP TO	PER CENT TOTAL NITROGEN	PER CENT MOISTURE
°C.		
110	0.0035	0.336
120	0.0042	0.220
130	0.0056	0.183
140	0.0070	0.158

*Effect of the temperature used in making on the keeping quality of ghee.* A comparison was made of the keeping qualities of four lots of ghee prepared by heating to four different temperatures—110°, 120°, 130°, and 140°C. The ghee made with the two lower temperatures developed a slight off condition at both room temperature and cooler temperature, while that made with the two higher temperatures remained satisfactory during the holding period of twenty-three weeks. The off condition was not at all suggestive of the cheesy condition noted in a number of lots of deteriorated ghee.

The heated flavor and odor of the different lots of ghee seemed to vary somewhat and be proportional to the temperatures used in the preparation.

The four lots of ghee were examined for the total nitrogen and

TABLE 2  
The constants of normal and abnormal ghee and butter fat before and after storage

SAMPLE	EXISTING CONDITION	QUALITY					CONSTANTS							
		When fresh	After 3 weeks	After 6 weeks	After 8 weeks	After 10 weeks	Acid value	Iodine number		Reichert-Meisel number	Melting point		Refractive index at 40°	
								Fresh	2 months after		Fresh	2 months after	Fresh	2 months after
Normal ghee	Cooler temperature	Good ghee odor	O. K.	O. K.	O. K.	O. K.	0.41	0.53	34.5	28.0	27.9	30.8	941.0	41.4
	Room temperature in dark	Good ghee odor	O. K.	O. K.	O. K.	O. K.	0.41	0.53	34.5	28.0	27.7	30.8	941.0	41.5
	Room temperature in light	Good ghee odor	O. K.	O. K.	O. K.	Slightly tallowy	0.41	0.57	34.5	28.0	27.9	30.8	941.0	41.4
Butter fat	Cooler temperature	Good butter odor	O. K.	O. K.	O. K.	O. K.	0.42	0.46	35.0	28.1	27.6	30.7	941.5	41.5
	Room temperature in dark	Good butter odor	O. K.	O. K.	O. K.	O. K.	0.42	0.48	35.0	28.1	27.6	30.7	941.5	41.4
	Room temperature in light	Good butter odor	O. K.	O. K.	O. K.	Very slightly tallowy	0.42	0.46	35.0	28.1	28.1	30.7	941.5	41.6
Ghee not strained	Cooler temperature	Good ghee odor	O. K.	O. K.	O. K.	O. K.	0.41	0.47	34.5	28.6	27.5	30.8	941.0	41.7
	Room temperature in dark	Good ghee odor	O. K.	O. K.	O. K.	O. K.	0.41	0.50	34.5	28.6	27.6	30.8	941.0	41.9
	Room temperature in light	Good ghee odor	O. K.	O. K.	O. K.	Very slightly tallowy	0.41	0.48	34.5	28.6	27.8	30.8	941.0	41.8

Ghee plus water	Cooler temperature	Good ghee odor	O. K.	O. K.	Off	Off and cheesy	0.41	0.45	34.5	35.5	28.0	27.1	30.8	30.9	41.5
	Room temperature in dark	Good ghee odor	O. K.	O. K.	Very cheesy and off	Very cheesy and off	0.41	0.73	34.5	35.2	28.0	27.3	30.8	30.7	41.5
	Room temperature in light	Good ghee odor	Slightly off	Slightly off	Off; mold growth	Off; mold growth	0.41	1.03	34.5	34.7	28.0	27.2	30.8	30.6	41.5
Ghee from poor butter	Cooler temperature	Ghee odor, but slightly off	O. K.	O. K.	O. K.	O. K.	0.78	0.78	34.3	34.7	27.4	27.9	31.9	31.8	41.7
	Room temperature in dark	Ghee odor, but slightly off	O. K.	O. K.	O. K.	O. K.	0.78	0.81	34.3	35.0	27.4	28.0	31.9	31.9	41.7
	Room temperature in light	Ghee odor, but slightly off	O. K.	O. K.	Very slightly talloxy	Very slightly talloxy	0.78	0.82	34.3	35.0	27.4	28.2	31.9	31.6	41.8

the moisture contents because of the possible relationship of the temperature of heating to the composition. The results secured are given in table 1 and show that with an increase in the temperature of the heating there was an increase in the per cent of total nitrogen but a decrease in the per cent of moisture; the increased moisture content of the ghee made with the lower temperatures, although comparatively small, may have been a factor in causing a greater deterioration.

### *Composition and fat constants*

*Nitrogen and moisture.* It would be expected that ghee would contain small amounts of protein and water because of their presence in butter. A number of lots of both ghee and butter fat were examined for these, but the amounts found were so small that considerable percentage error in the results is to be expected. With eight samples of normal ghee the per cent nitrogen varied from 0.000 to 0.019 and the per cent moisture from 0.039 to 0.166, while with three samples of butter fat the average values for nitrogen and for moisture were slightly lower.

*Fat constants of ghee before and after storage.* Certain fat constants were determined on (1) normal ghee, (2) butter fat, (3) unstrained ghee, (4) ghee with added water and (5) ghee made from poor butter, both before and after storage under different conditions. The results obtained are presented in table 2. From the data given it is evident that the constants on the fresh materials are in general agreement with the values usually found in the examination of butter. The principal change in the constants during holding was an increase in the acid value and in general this was most pronounced in the samples showing the greatest deterioration and small where the samples remained in a satisfactory condition. The iodine number, Reichert-Meissl number, melting point and refractometer reading showed no general change during the storage period.

Table 3 gives the fat constant on five samples of deteriorated ghee, two of which had been modified, together with the general defect noted. Here again the acid value shows the greatest change from what would undoubtedly be considered normal.

*Relationship of mold to the deterioration of ghee*

Molds of various types were generally present in ghee which was decidedly cheesy and accordingly it seemed desirable to study the relationship of molds to this condition.

TABLE 3  
*Constants on deteriorated ghee*

SAMPLE	QUALITY WHEN ANALYZED	CONSTANTS		
		Acid value	Iodine number	Reichert-Meissl number
Ghee plus curd.....	Very cheesy	1.16	31.8	28.3
Normal ghee.....	Cheesy	1.27	31.9	27.9
Ghee from poor butter in dark.....	Slightly off	1.01	31.5	28.0
Ghee from poor butter in light.....	Very tallowy	2.99	31.8	28.4
Ghee plus water.....	Very cheesy	1.23	31.7	28.3

TABLE 4  
*Observations and constants on normal and abnormal ghee, butter fat, and butter. All inoculated with mold*

SAMPLE	QUALITY				CONSTANTS AFTER 6 weeks		
	When fresh	After 1 week	After 4 weeks	After 6 weeks	Acid value	Iodine number	Reichert-Meissl number
Normal ghee	Good ghee odor	O. K.	O. K.	O. K.	0.81	36.5	27.35
Sterile butter fat	Slightly heated odor	O. K.	O. K.	Very slightly off	2.07	37.2	26.98
Ghee not strained	Good ghee odor	O. K.	O. K.	Very slightly off	1.57	36.8	27.50
Ghee plus water	Good ghee odor	Off; cheesy	Very cheesy	Very cheesy	9.18	36.4	27.00
Sterile butter	Slightly heated odor	Off; cheesy	Off; cheesy	Very cheesy	7.42	36.7	27.38

Various types of mold were isolated from cheesy ghee by pouring plates using whey agar. When these were inoculated into



butter which had been sterilized and cooled with agitation so as to reincorporate the water and curd, a cheesy condition commonly developed on standing in the dark at room temperature.

The influence of various factors on the growth of mold was then studied by preparing (1) normal ghee, (2) sterilized butter fat, (3) unstrained ghee, (4) ghee plus 16 per cent sterilized water and (5) sterilized butter, and cooling with agitation after which they were inoculated with mold. The samples were held in the dark at room temperature and examined at various intervals for quality; after six weeks some of the fat constants were determined and the results obtained are given in table 4. The data show that the butter and the ghee with the added water deteriorated rapidly and became cheesy; the normal ghee remained satisfactory, while the butter fat and unstrained ghee were very slightly off after six weeks. The principal change in the fat constants was an increase in the acid value and this increase was very pronounced in the case of the ghee with added water and the butter.

#### DISCUSSION OF RESULTS

From the results obtained it is evident that the ghee kept much better than the butter with which it was compared at both room temperature and cooler temperature. Butter fat, even when it had not been heated above 55°C., had keeping qualities much more nearly like ghee than like butter and this suggests that the elimination of water and curd were the factors giving ghee its keeping qualities, rather than the heat used in the manufacture. In accordance with this idea it was found that the addition of sterile water to ghee greatly decreased its keeping properties and that in one of three trials the failure to remove a part of the curd by straining resulted in a comparatively rapid deterioration. The general results obtained suggest that the presence of moisture is more important from the standpoint of deterioration than the presence of larger amounts of curd.

The exposure of ghee to diffuse light quite regularly resulted in the development of a tallowy condition and sometimes in

actual bleaching. This indicates the value of the practice of using earthenware or tin rather than glass for the storage of ghee.

The off flavor and odor in ghee made from poor butter show that the quality of the raw material, whether this be looked upon as milk, cream, or butter, is important with ghee just as it is with butter. The heat used in making ghee apparently does not remove or cover up off flavors and odors and it seems that considerable variation is to be expected in ghee made in small quantities because of variations in the quality of the raw material.

The higher the temperature used in making ghee, the more pronounced was the heated flavor and odor in the finished product. The higher temperatures also seemed to yield ghee with better keeping qualities; this may have been due to the less complete removal of the moisture with the lower temperatures.

The general results obtained on ghee suggest that its keeping properties are due to the composition. The elimination of water and curd makes conditions unfavorable for the growth of organisms and such hardy types as some of the molds have difficulty in developing.

The butter fat in a purified condition seemed to be quite resistant to the growth of microorganisms and other types of changes. Accordingly it would appear that in butter the constituents other than the fat are the important ones from the standpoint of deterioration. The conditions in butter are undoubtedly much more favorable for growth than in ghee and permit of the development of a greater variety of microorganisms than in the case of ghee where the growth seemed to be largely limited to molds.

The general findings suggest that the development of products made up almost entirely of butter fat may solve the problem of a food which can be used as butter and kept under severe temperature conditions. Such a product need not be prepared at high temperatures and thus the heated flavor and odor of ghee need not be present.

Analyses of ghee show that it contains only very small amounts of water or protein and its composition closely approaches that of butter fat.

During deterioration the principal change in the fat constants studied was an increase in the acid value. This suggests that the cheesy flavor and odor may be due to the liberation of certain fatty acids, such as caproic, caprylic and capric, which are believed to be important in accounting for the characteristic flavor and odor of Roquefort cheese. Definite changes did not occur in the iodine number, Reichert-Meissl number, melting point or refractometer reading, even when there were rather pronounced changes in flavor and odor. It seems probable that often the amount of any material required to give a definite off flavor and odor is so small it is difficult to detect the chemical change resulting in its formation.

#### SUMMARY

Studies on the keeping qualities of normal and abnormal ghee, butter and butter fat showed that ghee and butter fat kept much better than butter or ghee containing added moisture. This suggests that the composition is a big factor in the keeping qualities of these products. The addition of moisture seemed to favor the deterioration of ghee more than the addition of protein.

The flavor and odor of ghee which had developed a pronounced off condition were usually cheesy; in general the growth of mold accompanied the development of this condition.

The most definite change in the fat constants during the deterioration of ghee was an increase in the acid values.

# A METHOD OF AWARDING MARKS FOR UNIFORMITY OF LOW BACTERIAL COUNT IN CLEAN MILK COMPETITIONS\*

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In "Clean Milk Competitions" in England the cleanliness of a sample of milk is judged on three points, namely (1) the total number of organisms per cubic centimeter which grow on a standard nutrient agar, (2) the degree of infection by coliform organisms, as shown by the presumptive test and (3) the number of days which the milk remains sweet after milking when kept at a temperature of 60°F. Marks are awarded for the above points according to the scale suggested in the "Guide to the Conduct of Clean Milk Competitions (1926)" published by the Ministry of Agriculture. The competitions generally run for a period of six months, milk samples being taken at fortnightly intervals. The total number of marks awarded to a competitor under the three headings is in general taken to be an index of his ability to produce clean milk.

In this system of marking it will be noted that no account is taken of the consistency of cleanliness of successive examples of any one competitor. The graphs given in figures 1, 2, 3, and 4 show the percentage marks<sup>1</sup> awarded for each sample sent in by twelve competitors in a recent "Clean Milk Competition." From a study of these graphs it will be seen that there are cases in which the results of the samples sent in show extraordinary variations, due obviously to a disregard of the smaller, yet none the less important, details of clean milk production. Such a case is illustrated in the graph of competitor 2 (fig. 1). Here the competitor gained a 100 per cent of the marks for the fifth sample, yet on two occasions his samples were so poor that

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<sup>1</sup> These marks are also given in table 1.

no marks were awarded. On the other hand, there are cases in which, as the competition proceeds, the samples show consistently low bacterial counts, in some instances gradually decreasing, thus indicating a gradual improvement. The graphs of competitors

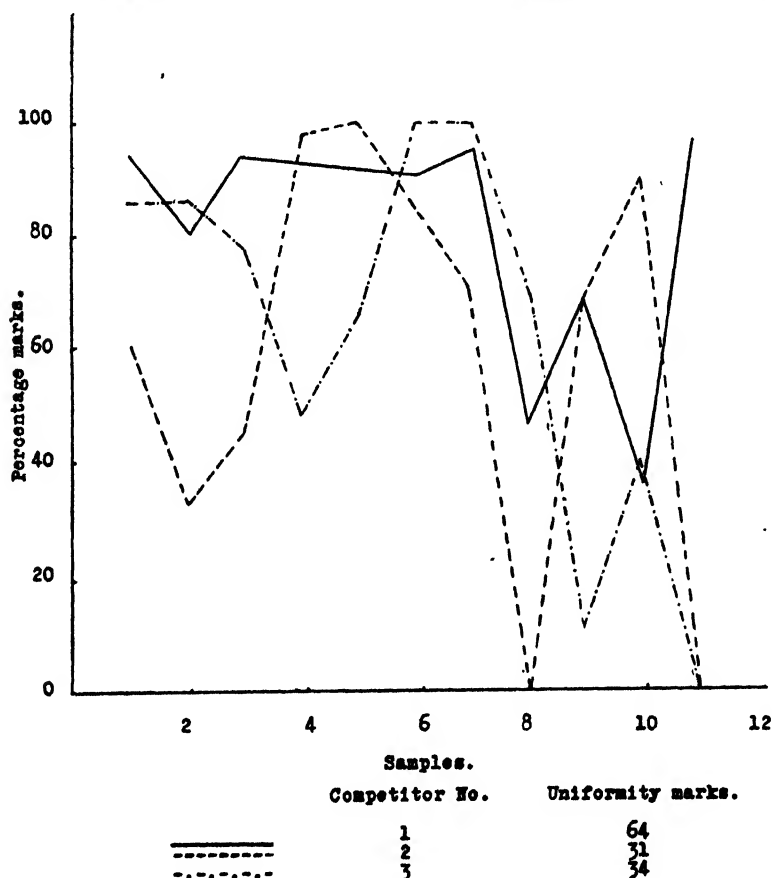


FIG. 1. GRAPH OF THE RESULTS OF COMPETITORS NOS. 1, 2 AND 3, SHOWING THE PERCENTAGE MARKS AWARDED FOR EACH SAMPLE

5 and 12 (fig. 4) for example, give almost a straight line. Nevertheless, even where the conditions under which the milk is produced are apparently beyond reproach an occasional high bacterial count may be met with, such as may be caused by a slight

inflammatory condition of the udders of one or two cows. The eighth sample sent in by competitor 9 (fig. 3) suggests such a case. Again, there are cases which, although the bacterial counts are comparatively high, show some degree of consistency, as for example, competitor 10 (fig. 3).

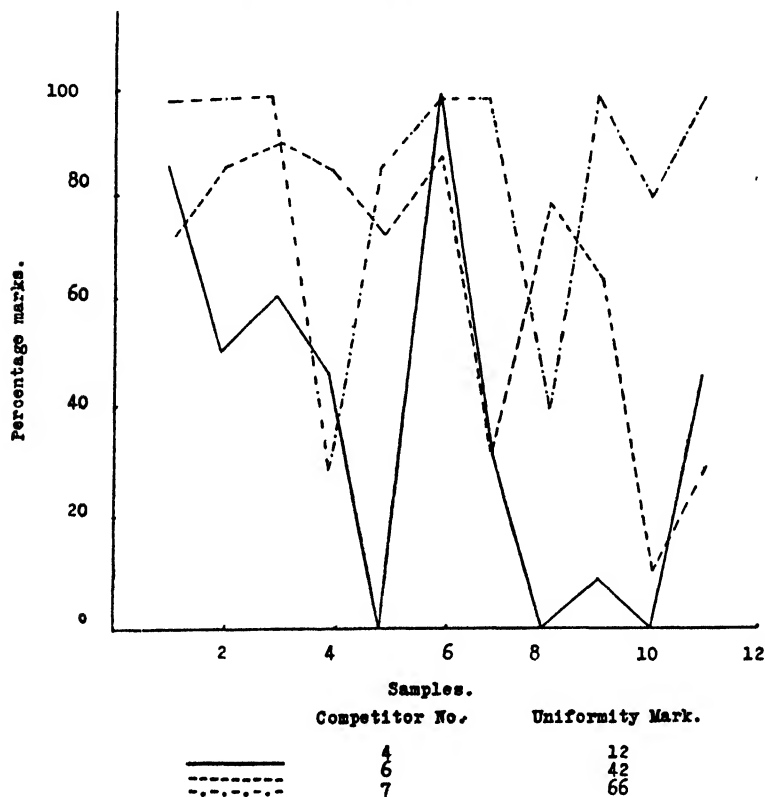


FIG. 2. GRAPHS OF THE RESULTS OF COMPETITORS NOS. 4, 6 AND 7, SHOWING THE PERCENTAGE MARKS AWARDED FOR EACH SAMPLE

It seems very desirable therefore, in view of the great variation which exists in the bacterial counts and keeping quality of successive samples sent in by any one competitor, to devise some method by which the larger number of marks is awarded to those competitors who consistently produce milk of a high bacterial

standard. A method which was adopted in the Yorkshire "Clean Milk Competition" for 1926 for solving this problem is as follows:

Marks are given for bacterial count, keeping quality and B. coli contamination in accordance with the scales suggested in the

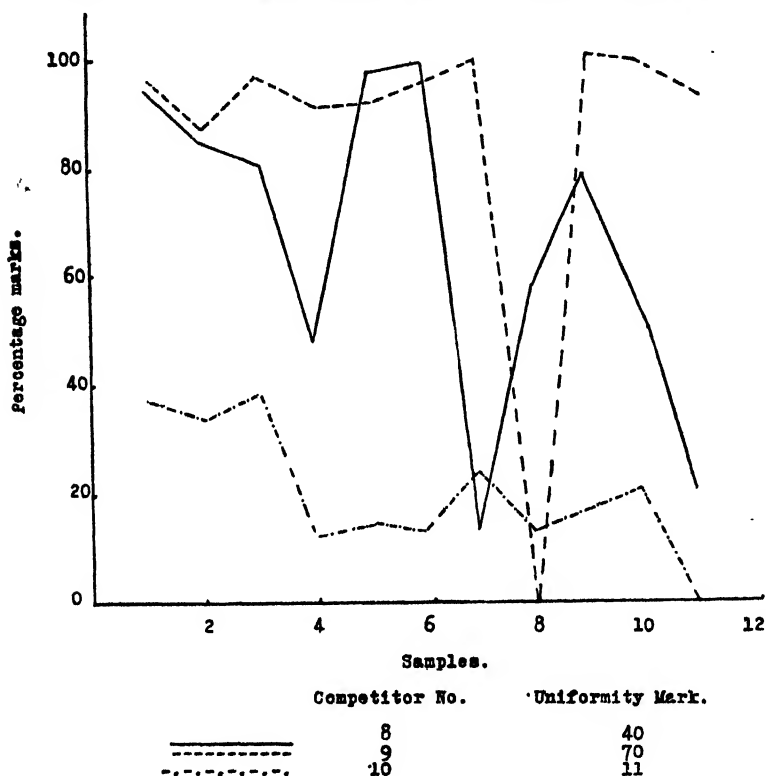


FIG. 3. GRAPHS OF THE RESULTS OF COMPETITORS NOS. 8, 9 AND 10, SHOWING THE PERCENTAGE MARKS AWARDED FOR EACH SAMPLE

"Guide to the Conduct of Clean Milk Competitions." The total marks for each sample is reduced to percentage (see table 1) and the average percentage mark of each competitor's samples determined. The mean variation of the percentage mark of each sample from this average percentage mark is then calculated.

The maximum marks awarded for uniformity is one hundred.

The average percentage mark of each competitor, as calculated above, represents the portion of the uniformity marks which are allocated for lowness of bacterial count and good keeping quality. The mean variation from the average percentage mark of each competitor represents the portion of the *uniformity marks* which

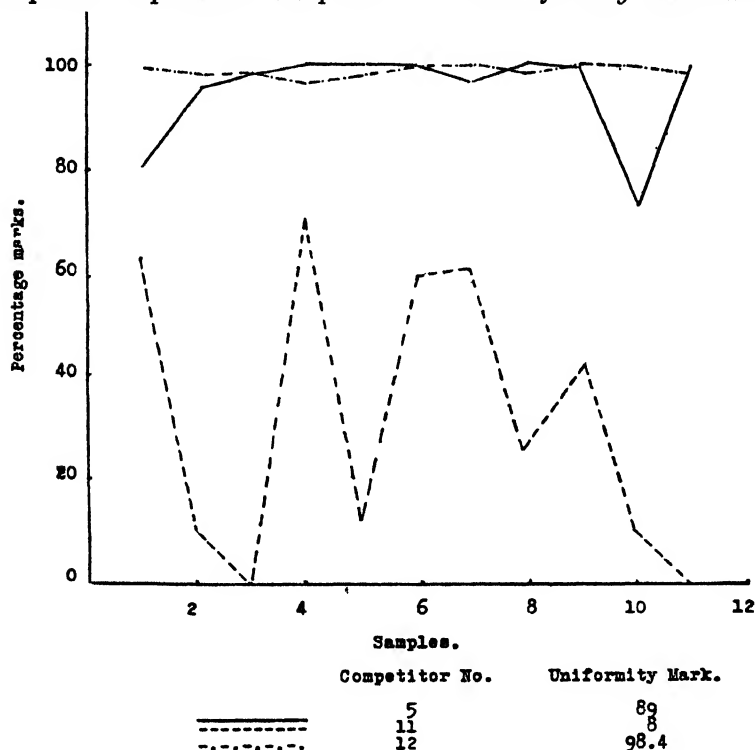


FIG. 4. GRAPHS OF THE RESULTS OF COMPETITORS NOS. 5, 11 AND 12, SHOWING THE PERCENTAGE MARKS AWARDED FOR EACH SAMPLE

are allocated for consistency of bacterial count and keeping quality.<sup>1</sup>

<sup>1</sup> It will be observed that the advantage of taking a hundred as the *uniformity mark* is that the portion of the *uniformity marks* which are allocated for lowness of bacterial count and keeping quality, and the portion of the *uniformity marks* allocated for consistency of bacterial count and keeping quality become numerically the same respectively as the average percentage mark and the mean deviation from the average percentage mark.



If now, the portion of the *uniformity marks* which are allocated for consistency of bacterial count and good keeping quality is deducted from the portion of the *uniformity marks* which are allocated for lowness of bacterial count, a figure is obtained which, while it compensates for low bacterial count and good keeping quality, penalizes for variations from the average bacterial count and keeping quality.

TABLE 1  
*Marks gained by competitors in a clean milk competition*

NUMBER OF COMPETI- TOR	PERCENTAGE MARKS AWARDED FOR EACH SAMPLE FOR TOTAL BACTERIAL COUNT, B. COLI, AND KEEPING QUALITY											AVERAGE PERCENT- AGE MARKS	MEAN VARIATION FROM THE AVERAGE	UNIFORMITY MARK	MARKS FOR EXAMINA- TION OF MILK (MAXIMUM, 800)	TOTAL MARKS FOR EXAMINATION OF THE MILK AND UNI- FORMITY
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11					
1	95	81	94	93	92	91	95	46	69	35	97	81	17.0	64.0	648	712.0
2	61	33	45	98	100	89	70	0	69	90	0	60	29.0	31.0	480	511.0
3	87	87	78	48	65	100	100	70	10	38	0	62	28.0	34.0	496	530.0
4	87	52	62	47	0	100	34	0	10	0	48	40	28.0	12.0	320	332.0
5	81	95	99	100	100	100	96	100	99	72	100	95	6.0	89.0	760	849.0
6	72	86	90	85	73	88	31	79	65	10	30	64	22.0	42.0	512	554.0
7	99	99	100	29	88	99	99	40	100	80	99	85	19.0	66.0	680	746.0
8	95	85	81	47	97	99	13	58	79	47	20	66	26.0	40.0	528	568.0
9	96	87	97	91	92	95	100	0	100	99	93	86	16.0	70.0	688	758.0
10	37	34	38	12	14	13	24	13	17	20	0	20	9.0	11.0	160	171.0
11	62	10	0	71	11	59	61	25	41	10	0	32	24.0	8.0	256	264.0
12	100	99	99	97	99	100	100	99	100	100	99	99	0.6	98.4	792	890.4

EXAMPLE, COMPETITOR 5 (TABLE 1)

The percentage marks awarded for bacterial count, B. coli contamination and keeping quality is as follows:

Sample 1.....	81
Sample 2.....	95
Sample 3.....	99
Sample 4.....	100
Sample 5.....	100
Sample 6.....	100
Sample 7.....	96
Sample 8.....	100

Sample 9.....	99
Sample 10.....	72
Sample 11.....	100
<hr/>	
Total percentage marks.....	1042
Average percentage mark.....	95

The variation of the percentage marks awarded for each sample from the average percentage mark is as follows:

Sample 1.....	(81-95) = 14
Sample 2.....	95-95 = 0
Sample 3.....	99-95 = 4
Sample 4.....	100-95 = 5
Sample 5.....	100-95 = 5
Sample 6.....	100-95 = 5
Sample 7.....	96-95 = 1
Sample 8.....	100-95 = 5
Sample 9.....	99-95 = 4
Sample 10.....	72-95 = 23
Sample 11.....	100-95 = 5
<hr/>	
Total variation.....	71
Average variation.....	6

The marks for uniformity, i.e., the difference between the average variation and the average percentage mark is  $95 - 6 = 89$ .

The *uniformity mark* therefore, which this competitor receives is 89.

It will be seen from the foregoing example and the figures given in table 1 that this method of awarding the *uniformity marks* gives (1) the highest number of marks to those competitors who have maintained a low bacterial count and good keeping quality, as in the case of competitors 5 and 12.

	AVERAGE PERCENTAGE MARKS	MEAN VARIATION FROM THE AVERAGE	UNIFORMITY MARKS
Competitor 5.....	95	6	89.0
Competitor 12.....	99	0 6	98.4

(2) A higher *uniformity mark* to those competitors whose average bacterial count and keeping quality, although of a slightly

lower standard, show a greater uniformity than do those competitors who actually have a lower average count and higher keeping quality but lack consistency. For example, the results of competitors No. 6 and 8 compared are as follows:

	AVERAGE PERCENTAGE MARKS	MEAN VARIATION FROM THE AVERAGE	UNIFORMITY MARKS
Competitor 6.....	64	22	42
Competitor 8.....	66	26	40

(3) The least number of marks to those competitors whose count and keeping quality are poor and not even consistently poor, as in the case of competitor 11.

The *uniformity mark* therefore is a figure which compensates for low bacterial count and good keeping quality, yet penalizes for variations from the average bacterial count and keeping quality.

# A METHOD OF OBTAINING CRUDE MILK SUGAR AND OTHER SOLIDS FROM SWEET WHEY\*

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The annual production of milk sugar (lactose) in the United States is from two to four million pounds, which is only a small part of what could be produced if the demand for it were greater. If the cost of recovering milk sugar from whey could be considerably lessened so that it could compete with other sugars on a more favorable price level, it would be a great help to those who are seeking new outlets for milk sugar. The purpose of this paper is to describe how this may be accomplished.<sup>1</sup>

Whey is the product remaining after the removal of most of the fat and casein from milk either in cheese making or in the manufacture of casein. Its composition varies with the methods employed in removing the fat and casein. A representative analysis is as follows: Water, 93.1 per cent; lactose, 5.0 per cent; proteins, 1.0 per cent; ash (mostly inorganic salts), 0.5 per cent; and fat, 0.4 per cent. The titratable acidity, measured as lactic acid with phenolphthalein as the indicator, varies from 0.10 per cent to about 0.75 per cent, depending on the product that is being made from the milk.

Nearly three-fourths of the mineral salts of milk pass into the whey. The proportion of some of the mineral constituents of milk which pass into the whey in the manufacture of cheddar cheese is given by Berry (1) as follows: Lime, CaO, 36.0 per cent:

\*Received for publication November 7, 1927.

<sup>1</sup> The process described in this paper is covered by the following: Weimar, A. C., Process of extracting soluble albumin from whey, U. S. Patent No. 1,381,605, June 14, 1921; Bell, R. W., Process of separating proteins and other matter from whey in soluble form, U. S. Patent No. 1,600,161, September 14, 1926; and Bell, R. W., Process for the manufacture of crude milk sugar, U. S. Patent No. 1,600,573, September 21, 1926. These patents have been dedicated to the public.

phosphoric acid,  $P_2O_5$ , 49.0 per cent; magnesia,  $MgO$ , 75.0 per cent; and potash,  $K_2O$ , 80.0 per cent.

Several billion pounds of whey are made available annually in this country in the production of cheese and casein. This is from two-thirds to three-fourths of the weight of the milk from which it is obtained. Since the percentage of solids in whey is more than one-half that of milk, it becomes apparent that the proper disposal of these solids is of great economic importance. Most of the whey is now fed to livestock or thrown away. The aim should be to make the solids directly available as human food.

#### PRESENT METHODS OF MANUFACTURING CRUDE MILK SUGAR<sup>2</sup>

Most of the milk sugar produced in the United States is recovered from grain curd casein whey. In the manufacture of grain curd casein, dilute hydrochloric acid is mixed with skim milk in such proportions as to precipitate the casein and to give the whey a hydrogen ion concentration of 4.1 in terms of pH, or a titratable acidity of about 0.47 per cent. After separation from the casein the whey is condensed, usually in double effect evaporators, to 18° Baumé and run into deep wells for heating to the boiling point, for neutralizing with lime to only a faint alkalinity to litmus paper, and for the settling of the coagulated proteins and insoluble salts. Some people prefer to neutralize the whey to a pH value not greater than 6.2 and to boil it to remove the coagulated proteins and insoluble salts before condensing. Advocates of this method claim that a more favorable medium for the crystallization of the sugar is obtained. When the 18° Baumé whey is neutralized to faint alkalinity to litmus paper, the claim is made that the insoluble material formed by neutralizing and boiling settles better than at a more acid reaction. In both methods the supernatant liquid is siphoned off and run through filter presses. The whey is pressed from the precipitated material at the last stage of the filtering operation.

The filtrate is condensed in a vertical type pan by first filling

<sup>2</sup> For technical information concerning milk sugar see "Lactose: A review" by E. O. Whittier, *Chem. Reviews*, ii, 85 (1925).

the pan with the sugar syrup and condensing in such a way that the crystals which have formed will serve as nuclei for obtaining and speeding up suitable crystal growth. The last part of the condensing operation, known as the graining process, is carried on slowly to insure the proper formation of sugar crystals. When all the syrup has been drawn into the pan and a concentration sufficient to give a reading of 37.0° to 40.0° Baumé has been attained, the material is removed from the condenser to large crystallizing vats. After slow stirring for one or more hours, accompanied by gradual cooling to approximately 70.0°F., the crystallized sugar is removed from the mother liquor by means of a sugar centrifugal. In one large plant the 37.0° Baumé syrup is left in wooden vats at room temperature for four days to obtain satisfactory crystallization. The filtrate from the centrifugal may be used to obtain more sugar by a second crystallization, it may be dried and used as part of a poultry feed mixture, or it may be discarded.

The crude sugar is washed in the centrifugal and the wash water is added to the next batch of whey. If the crude sugar which contains 85.0 to 90.0 per cent lactose is not produced at a refinery it must be dried on trays in hot air tunnels previous to shipment for refining. The yield of washed and dried crude sugar from one hundred pounds of whey does not exceed three and three-fourths pounds. The filtrate which comes from the press following the removal of coagulated proteins and insoluble salts may be passed by gravity through an activated charcoal filter for the removal of the pigment which gives it a yellow color, again filtered, and evaporated. The sugar recovered from it needs only to be washed, dried, and ground to produce a refined product.

About three-fifths of the proteins of whey are coagulable. The prevailing method of removing them from the whey renders them insoluble. When these proteins, together with certain insoluble salts which make up the sludge from the filter press, are dried they are sold for four or five cents per pound, a price which hardly pays for the cost of drying and handling.

## EXPERIMENTAL

This paper describes a process for the recovery of crude milk sugar from fat-free, sweet whey in which the boiling and filter-pressing operations for the removal of coagulable proteins and insoluble salts have been eliminated. It not only has certain advantages of increased yield and greater efficiency of operation over the methods now in use; but it also makes possible the recovery, in a powdered and soluble form, of the solids in the whey other than those removed as crude milk sugar.

Swiss-cheese whey was used in the experimental work because it seemed that the probability of recovering most of the lactose without first removing the coagulable proteins would be increased with a type of whey which was sweet, low in salt content, and very uniform in composition. Swiss-cheese whey was available daily as a result of other experimental work. It had a pH value of  $6.4 \pm 0.05$  or a titratable acidity of 0.10 to 0.115 per cent. It was composed of ash, 0.5 per cent; fat, 0.76 per cent; lactose, 5.2 per cent; protein, 0.99 per cent; and water, 92.49 per cent. A lactose content in excess of 5.0 per cent was not expected since more than 5.0 per cent lactose in whey is considered high.

The fat was removed with a suitable separator in order to obtain a more favorable material for the recovery of the sugar. A two-foot vertical type pan and an inclined continuous evaporator were available for condensing. In the latter one thousand pounds of whey could be concentrated to a nine to one ratio in one hour.

## EFFECT OF THE REACTION OF THE WHEY

Variations in the concentration and methods of cooling the fat-free whey did not prevent the formation of slime in the sugar centrifugal. Many types of filter cloths of different weaves were used without remedying the difficulty. The slime contained proteins, lactose, and salts in about equal proportions; and when obtained after a thorough whirling of the centrifugal it had a total solids content of 25.0 per cent. The elimination of slime and the consequent ability to obtain a cleaner and quicker separation of the sugar crystals from the mother liquor were made possi-

TABLE 1  
Effect of neutralizing sweet whey on the formation of slime

RUN NUMBER	TITRATABLE ACIDITY	TOTAL SOLIDS IN CONDENSED WHEY	YIELD OF CRUDE SUGAR FROM WHEY	YIELD OF LIQUOR FROM WHEY	COMPOSITION OF CRUDE SUGAR				COMPOSITION OF LIQUOR				REMARKS
					Ash	Pro- tein	Water	Lac- tose	Ash	Pro- tein	Lac- tose	Total solids	
1	0.11	61.10	5.0	4.8	2.9	4.7	17.0	75.6	7.0	13.3	17.8	38.1	Slime present. Whey not neutralized enough. Tried to force liquor through by extra centri- fuging. Poor quality sugar
2	0.08	61.44	4.6	3.2*	...	.....	.....	.....	4.30	14.70	21.74	40.74	Slime prevented separation of liquor from sugar. Small crystals in condensed whey. Whey not neutralized enough. Sugar very poor
3	0.06	62.10	4.7	5.0	2.05	2.36	11.00	84.50	7.65	14.3	17.05	39.0	Sugar poor in quality and not dry enough. A little slime
4	0.04	62.41	5.0	5.6	1.14	1.75	6.33	91.20	5.04	14.08	21.24	40.36	Sugar excellent. Run satisfactory. No slime

\* Some lost.



ble by neutralizing the whey to a pH of 7.3 or a titratable acidity of 0.04 per cent. Neutralizing beyond this point was not necessary, but it was essential that the reaction mentioned be attained in order to be certain that slime would not form. A 5.0 per cent solution of sodium hydroxide was used for neutralizing, care being taken in mixing it with the whey not to cause local concentration of the alkali with consequent precipitation of proteins and salts.

In table 1 typical data are given showing the effect of the reaction of the whey on the formation of slime in the centrifugal and the composition of the unwashed crude sugar and liquor. As the quantity of slime became less the quality of the sugar improved. As soon as the liquor ceased to flow from the centrifugal samples of crude sugar and liquor were taken for analyzing. Other conditions being satisfactory, the success of the process depended upon the proper reaction of the whey before condensing. The figures given in tables 1 and 2, for the per cent of ash in the liquor are, of course, considerably less than the salt content. The ratio of ash to salts is not known. In analyzing for ash, proteins, and lactose in the liquor it was found that the sum of their contents was less than that of the total solids by nearly 4.0 per cent. The results for lactose were low, because of incomplete precipitation of proteins in the analytical procedure. The amount of lactose in the liquor, as it is given in the tables, was found by subtracting the sum of the ash and protein contents from the total solids content. The quantity of salts in the liquor, then, was somewhat greater than that shown by the figures given for ash, and the lactose percentage was somewhat less than indicated.

#### EFFECT OF CONCENTRATION OF SOLIDS IN THE CONDENSED WHEY

The neutralized whey was warmed in a stream jacketed container to not more than 60°C. (140°F.) before condensing. Cold whey could have been drawn directly into the evaporator with just as satisfactory results. A Baumé reading of 32.0° at 50°C. (131°F.), corresponding to a total solids content of approximately 62.0 per cent, was found to be the best point at which to strike the batch. It was better to exceed the concentration given than not to attain it, because at lower concentrations there was a

definite decrease in the yield of crude sugar. If the total solids content exceeded 64.0 per cent, the resulting product was sometimes not sufficiently fluid to be handled easily at 0°C. or lower. The proper concentration to attain will vary with the type of whey and the temperature used for crystallizing the sugar. Slime formed in many cases if the whey was overcondensed so that it had to be diluted to insure its being sufficiently fluid to pour after crystallization of the sugar.

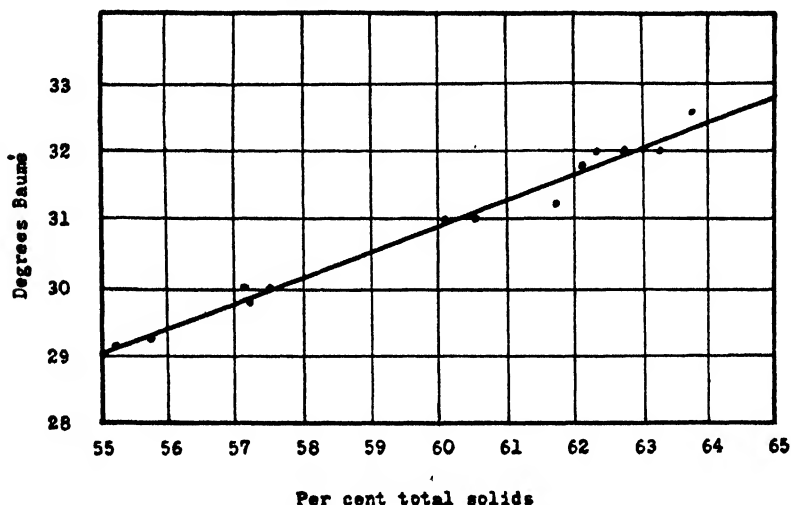


FIG. 1. THE RELATION OF THE BAUMÉ READING TO TOTAL SOLIDS IN CONDENSED FAT-FREE WHEY

Temperatures, 46.0° to 50.0°C., inclusive

It is necessary that a comparatively simple, fairly accurate and quick method be known for determining the proper time at which to strike the batch. An operator will soon learn to judge very closely by the appearance of the material in the pan when the desired concentration has been reached. Figure 1 gives the relation between the Baumé readings taken at temperatures between 46° and 50°C., inclusive, and the total solids content of neutralized and condensed fat-free Swiss-cheese whey.

The results show that the variation in total solids for the same

TABLE 2  
Effect of total solids content of condensed whey on the yield of crude milk sugar and liquor from 100 pounds of whey

RUN NUMBER	TITRATABLE ACIDITY OF NEUTRALIZED WHEY		TOTAL SOLIDS IN WHEY		CONDENSED WHEY		YIELD OF CRUDE SUGAR		YIELD OF LIQUOR		COMPOSITION OF CRUDE SUGAR				COMPOSITION OF LIQUOR				REMARKS
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	Ash	Protein	Water	Lactose	Ash	Protein	Lactose	Total solids	
1	0.045	53.22	3.9	8.3	per cent	per cent	1.52	1.55	8.35	89.00	3.52	10.86	21.12	35.50	per cent	per cent	per cent	per cent	Sugar excellent. Yield low. Whey not condensed enough
2	0.04	59.10	4.6	6.0	per cent	per cent	1.31	1.89	7.67	89.20	4.31	13.56	19.65	37.52	per cent	per cent	per cent	per cent	Same as no. 1
3	0.04	61.86	5.3	5.4	per cent	per cent	1.34	1.49	6.15	91.10	5.10	15.27	19.90	40.27	per cent	per cent	per cent	per cent	Sugar excellent. Run satisfactory.
4	0.04	64.81	5.4	5.1	per cent	per cent	1.97	2.76	9.73	85.54	5.06	15.19	20.29	40.54	per cent	per cent	per cent	per cent	Sugar good but inferior to No. 3. Total solids in condensed whey too high

Baumé reading and approximate temperature may be  $\pm 0.5$  per cent. Changes in methods of concentrating the same type of whey will cause different Baumé readings, even when the temperature and total solids content are the same, principally because of variations in the amount of sugar that has crystallized.

Table 2 shows the effect of the total solids content of the condensed whey on the yield of liquor and unwashed crude sugar.

#### EFFECT OF TEMPERATURE, SEEDING, AND STIRRING ON CRYSTALLIZATION

Some important factors controlling the crystallization of lactose from whey are concentration, temperature, seeding and stirring. The effect of the concentration on the yield has been given.

Satisfactory crystallization of the sugar was obtained by first cooling the condensed whey to  $25^{\circ}\text{C}.$ , by stirring it in cans that were surrounded by cold water and then leaving it for 42 hours in a cooler that had a temperature of  $4.0^{\circ}\text{C}.$  This procedure was employed in the experiments recorded in tables 1 and 2. Satisfactory crystallization of the sugar was also obtained by leaving the condensed whey for eighteen hours in a refrigerator that had a temperature of  $0^{\circ}\text{C}.$  In these experiments the condensed whey was not stirred. Lower temperatures and longer periods for crystallizing did not increase the yield. Cooling the condensed whey with slow stirring to  $0^{\circ}\text{C}.$ , or lower, and holding for more than 18 hours has the advantage of giving a larger and drier type of crystal. It increases the likelihood of obtaining good results. Refrigerating at higher temperatures and for shorter lengths of time gave a less desirable material for centrifuging and a decrease in the yield of crude sugar. In the laboratories of the Bureau of Dairy Industry yields of 4 pounds of sugar of 85 per cent purity have been obtained from 100 pounds of Swiss-cheese whey by cooling the condensed whey, without stirring, to  $15.0^{\circ}\text{C}.$ , and holding it at that temperature for eighteen hours. In these experiments the whey was condensed to  $36.0^{\circ}$  Baumé at  $46.0^{\circ}\text{C}.$

In the crystallization from wheys in which the concentration of solids is high, the lactose will always be in the labile state, nuclei will induce crystallization, and copious seeding should

result in a rapid general formation of crystals. The "graining" process practiced commercially is therefore desirable in obtaining a favorable development of crystals. It is important that crystallization take place as much as possible in the pan. This step has been more successful in the vertical type evaporator which was used than in the continuous unit. In general, the methods now employed in commercial practice for obtaining a satisfactory crystal growth also apply to the recovery of crude milk sugar from neutralized and condensed fat-free sweet whey.

#### PROPERTIES OF THE LIQUOR AND POWDER

The liquor (filtrate) was not suitable for condensing to obtain a second crop of crystals.

Due to the brownish color of the liquor it was impossible to determine its acidity by a color indicator method. On attainment of equilibrium, that is, as soon as the liquor had been warmed to 24°C. and become free from air, a potentiometric titration showed that its pH value was 0.8 to 1.0 unit less than the pH value of the neutralized whey from which it was obtained.

When held at temperatures low enough to inhibit growth of microorganisms the liquor kept very well. The viscosity increased with time, and if the liquor remained at room temperature for a few days a very definite jellying became apparent.

The liquor was dried by the spray and vacuum drum processes. The appearance and solubility of the powder was governed largely by the method of drying. The powder was light yellow in color, depending on the fineness of the particles and the care taken to prevent unnecessary heating. It emulsified well when dissolved in water and beaten with glucose and cane sugar. The lactalbumin in the powder did not have the property of coagulating with the taking up of water as does egg albumin when it is heated.

Nutrition investigators have done a great deal of work on the food value of casein and lactalbumin. Lactalbumin is a perfect protein in that all of its amino acids are readily utilized in nutrition. The presence in the powder of about two parts of lactalbumin to one part of casein leaves no doubt as to the nutritive

value of the protein constituents, since the casein furnishes cystine which is considered by some to be lacking in lactalbumin. The water soluble vitamin B is found in milk and its products in great abundance. It has been found by long and careful research that this vitamin is not appreciably affected by the usual manufacturing processes used in the handling of milk and its products (2). It is probable, therefore, that the powder is high in vitamin B content. The other ingredients are milk sugar and the soluble salts of milk, thereby making it a desirable ingredient for modified milks. Suggestions which may lead to the manufacture and use of this product will be welcomed.

#### APPLICATION TO DIFFERENT TYPES OF WHEY

The process as described for Swiss-cheese whey has been applied on an experimental scale to Cheddar-cheese whey and on a semi-commercial scale to Roquefort and Pineapple-cheese whey. The results obtained, although not so satisfactory as those with Swiss-cheese whey, indicate that whey having a titratable acidity of 0.2 per cent or less may be used with good results. Whey having an acidity greater than 0.24 per cent immediately following its separation from the curd does not give satisfactory results either as to the yield or as to the quality of the sugar.

Using rennet casein whey the process was successfully demonstrated on a semi-commercial scale to a representative of one of the large manufacturers of milk sugar. He stated that the unwashed crude sugar obtained during the demonstration was cleaner and freer from impurities than that made by present commercial methods. The crystals were large and had a gritty feel, due to their sharp corners, thereby indicating that they were of good quality. Other things being equal, fewer impurities will be present in the capillary film in large crystals per unit weight of sugar than in small crystals.

The use of rennet casein whey is to be preferred because the salt content is low and the lactose percentage has not been lessened due to fermentation.

In a later paper it will be shown that whey having a titratable acidity greater than 0.24 per cent immediately following the

removal of the curd gives a precipitate when neutralized with a normal solution of sodium or calcium hydroxide, and that this neutralization precipitate is composed of protein material and tri-calcium phosphate, the proportion depending upon the reaction of the whey immediately following the removal of the curd.

#### DISCUSSION OF RESULTS AND THEIR APPLICATION

A method has been described for obtaining from whey of 0.2 per cent acid content or less, crude milk sugar and a soluble powder composed of the solids in the fat-free whey other than those removed as crude sugar.

The process now employed in commercial practice for the recovery of milk sugar from whey has been shortened by eliminating expensive boiling and filter-pressing operations. Only one condensing process is needed to obtain a Baumé reading of approximately 32.0°, whereas two operations are necessary in the usual procedure where a concentration of 18.0° Baumé is first attained, followed by a second condensing operation to bring the material from the filter press to test 37.0° to 40.0° Baumé.

Estimating the yield of washed and dried crude sugar to be 4 per cent and of powder 1.5 per cent, 5.5 pounds of finished product per 100 pounds of whey become available for sale. At 10 cents per pound for the crude sugar and at the same price for the powder, the returns per 100 pounds of whey are 55 cents. Two products that will return a profit are obtained instead of one as is now the case in the manufacture of milk sugar.

The market for milk sugar is limited. Improvements in the manufacturing process leading to a substantial reduction in its cost will make the development of new outlets easier. It is hoped that the results of this paper will be an aid toward that end and therefore bring about a better and more extensive utilization of the solids in whey.

#### REFERENCES

- (1) BERRY, R. A.: Jour. Agr. Sci., xiii, 214 (1923).
- (2) KENNEDY, C.: Vitamins in preserved milks. Proc. World's Dairy Congress, i, 198 (1923).

# GASSY FERMENTATIONS IN REHEATED OR PROCESSED CHEESE PRODUCTS CONTAINING PIMENTOS\*

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Manufacturers of cream cheese, loaf cheese, and cheese spreads experience considerable difficulty from gassy fermentations when pimentos are incorporated in these products. The same difficulty is experienced with other food products that contain pimentos. In most cases bacteria are responsible for the fermentations. In unheated products, however, such as cream cheese, the fermentation may be due to the presence of yeasts. This paper deals particularly with gassy fermentations in reheated or processed cheese products.

Reheated or processed cheese products are particularly favorable media for the growth of bacteria. There is present an abundance of easily available protein and inorganic salts. The water which is added to these products in the manufacturing process increases the moisture content beyond that of the cheese from which they are made; in cheese spreads the moisture is often as high as 50 per cent. The salts that are usually added to bring about the necessary homogeneity in the products increase their buffer content accordingly. The incorporation of pimentos supplies a fermentable carbohydrate. The bacteria which have survived the manufacturing process or have found their way into the product from equipment or containers find, therefore, favorable conditions for growth. This is particularly true in the case

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of the anaerobic organisms, since the products are packaged while hot and therefore contain very little free oxygen.

Certain anaerobic spore-forming bacteria are usually responsible for the gassy fermentations in reheated cheese products to which pimentos have been added to obtain variety. The source of these bacteria may be the cheese from which the products are made. Spore-forming anaerobes have frequently been isolated from cheese and in the spore state these bacteria survive the temperatures of processing. Because of the destruction by the heating process of practically all other types of organisms in the cheese, germination of the spores and subsequent growth of the anaerobes proceeds with little competition. Certain cocci which are known to survive the heating process multiply quite rapidly in the finished cheese product but these apparently do not interfere with the development of gas-producing bacteria.

A spore-forming anaerobic organism, capable of producing a vigorous gassy fermentation in pasteurized cheese products, has been found in both foreign- and domestic-packed pimentos. This is contrary to the experience of Warren (1) who reported that he found canned pimentos to be sterile. It is therefore of utmost importance that the pimentos be sterilized under pressure before they are incorporated in the food product, in order that inoculation from this source may be prevented. The sterility of the pimentos may easily be determined by adding a small quantity of them to freshly sterilized and cooled milk and incubating it for several days at a temperature of 37° to 40°C.

The containers in which the products are packed may sometimes be the source of gas-producing bacteria, in spite of the fact that they have been passed through a washing process. Contamination from this source can easily be determined by filling some of the containers with freshly sterilized, quickly cooled milk, capping and setting in a warm place to incubate.

Unclean equipment as a source of contamination should be too familiar a subject to warrant discussion in this paper.

It is possible, then, to protect reheated or processed cheese products from contamination with gas-producing bacteria from every source except the cheese itself. Contamination from this

source might also be controlled and the gassy fermentation prevented if it were economically possible to use only the best quality of cheese. Inasmuch as a certain amount of cheese of poor quality is always used, the possible presence of gas-producing bacteria must be accepted. Sterilization of the manufactured products is obviously impossible. It remains, therefore, to render the products less favorable as media for the development of the gas-producing bacteria if the fermentation is to be prevented. This was attempted by removing the fermentable carbohydrate from the pimentos. In the absence of a fermentable sugar a gassy fermentation is not likely to take place. Certain anaerobic gas-producers can utilize lactates with the production of gas; but this rarely occurs unless very young cheeses are used.

Both foreign- and domestic-packed pimentos were used in the experiments carried out. The pimentos were washed in double their weight of tap water three times in thirty minutes. They were then allowed to stand in fresh water for about 18 hours. The pimentos were then rinsed in fresh water, ground and sterilized.

A quantity of plain cheese spread was warmed until it reached a semifluid consistency. It was then divided into two equal portions. To one portion 10 per cent of sterilized washed pimentos was added, and to the other 10 per cent of ground, sterile unwashed pimentos. Both portions were put in small glass jars, capped and left standing in a warm place. Gas developed in the jars of cheese to which the unwashed pimentos had been added, but no gas was evident in the product which contained the washed pimentos.

This experiment has been repeated a number of times, with different batches of cheese. The quantity of the pimentos used has varied from 4 to 10 per cent. In every case in which gas developed in the cheese containing unwashed pimentos, the same cheese containing washed pimentos was free from gas.

It is true that some of the flavor and color of the pimentos are lost in the washing process. It is believed, however, that the washing was excessive and can be considerably lessened, in which case the flavor and color of the pimentos would be little affected.

This method has not been tried out on a factory scale; but the results obtained in laboratory experiments have been so favorable that successful application to factory practice is believed to be assured.

Although the studies reported have been confined to reheated cheese products, the findings are applicable to all food products in which pimentos are incorporated which are subject to a gassy fermentation.

#### SUMMARY

1. It has been found that washing the pimentos to remove the fermentable sugar they contain prevents the development of a gassy fermentation in reheated or processed cheese products in which pimentos have been incorporated.

#### REFERENCE

- (1) WARREN, D. H.: Jour. Dairy Sci., ix, (1926).

# THE VALUE OF SILAGE IN THE EXPERIMENTAL RATION\*

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The best method of attack for many nutritional studies of dairy cows is to find a ration that will allow the cows to give as nearly as possible their maximum milk yield year after year with no interruption in reproduction. Such a ration when found should be fed for one lactation period, and the data obtained during this period would become the base-line measure of the producing ability of each cow to be studied. Later the various nutritional factors should be tried out separately and the results measured quantitatively, using the results of the base-line lactation as a standard. For obvious reasons such a basal ration should be as simple as possible.

Nutritional studies of this nature are being made at the Bureau of Dairy Industry experiment station at Beltsville, Maryland. The basal ration is changed from time to time when trial proves certain constituents unsatisfactory. Thus the ration may be simplified or a certain constituent may be replaced by one which appears to be better.

The question as to whether or not silage should be used in such a ration is of considerable importance. Investigators who conduct comparative feeding trials where the balance of nutrients is rather carefully made, as well as those who carry on complete balance experiments, know how difficult it is to obtain accurate data when silage is used. Silage is greatly variable in nutrient content in different parts of the same silo, but it is even more so from year to year. The presence of cobs makes the taking of small accurate samples extremely difficult. Thus the feeding of silage in the balance-experiment ration greatly increases the

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labor involved and decreases the accuracy of the results. In the long-time feeding trials, involving ration comparisons, the variability of the quality of the silage when fed in large amounts may be great enough to make the results very inaccurate.

In April, 1922, many cows in the herd at Beltsville showed marked signs of digestive disturbance, such as off feed, scouring, and sudden drop in milk yield, which were due in all probability to some peculiar mold in the silage. When the silage feeding was discontinued, recovery was almost immediate. To forestall a repetition of these difficulties very few cows in the nutrition herd have received silage since that time. The basal ration has been simplified by discontinuing silage feed, and the results show that production has not been reduced.

In the latter part of 1925 it was decided to carry out some carefully controlled feeding trials to decide conclusively whether the basal ration of alfalfa hay and a grain mixture was producing as much milk as would a similar ration which included silage. These trials seemed necessary, since silage feeding had been discontinued in spite of the traditional teaching that some succulence, that is, feed retaining its plant juices, should be included in the ration.

#### SOME RESULTS AT OTHER STATIONS

Investigations concerning the value of silage in the ration fall roughly into the following classes: (a) early work carried on about 1890, in which corn-silage feeding was largely compared with corn-fodder feeding; and (b) later work with more adequate rations in which results of silage feeding were usually compared by substituting silage for a portion of the legume hay of the non-silage ration.

Even in the early work carried out at the Wisconsin (10, 15) and Vermont (7) Stations in which the cows were frequently fed as much silage and corn fodder as they would eat, the results varied from those slightly in favor of corn-fodder feeding to those considerably in favor of corn-silage feeding. Frequently, however, the consumption of silage, because of its greater palatability, was considerably larger than that of corn fodder. In two

cases at the Vermont (11) and Maine (2) Stations where silage was compared with mixed or timothy hay the silage ration produced more milk, though in one experiment the difference in nutrients consumed nearly accounted for the difference in yield. In most of these experiments, as well as in those of the second group, the periods were short, varying from twenty to thirty days.

The more recent trials are mentioned singly but briefly. Those in favor of the silage ration are stated first. At the Ohio Station (13) five cows in each of two groups seemingly ate about the Savage requirements and the silage group produced more milk, but as the groups were not reversed the evidence is not conclusive. Cows fed considerably over requirements for two brief periods by reversal at the Montana Station (5) produced about 2 per cent more milk on the silage ration. The production of twelve cows fed oat and pea silage versus mixed hay for twenty-one day periods by reversal at the Pennsylvania Station (3) showed a very large difference in favor of silage, though on calculation it appeared that both rations were about 20 per cent short of the Savage requirements. The silage ration in the experiments at the Utah Station (4) gave less than 2 per cent increase in production though this ration contained about 1 per cent more nutrients. The Purdue University (8) results showed about 10 per cent more production on the silage ration, which, however, contained about 5 per cent more nutrients.

The Nebraska Station (12) reports about 2 per cent greater production on the non-silage ration, but no substantiating data were given. At the Arizona Station (14) seven cows divided into two groups of four and three were fed, by reversal, a ration of 30 pounds of alfalfa hay and a ration of 20 pounds of the hay and 35 pounds of silage, no grain being fed. The hay ration produced about 2 per cent more milk, though the silage ration must have contained at least 6 per cent more nutrients. Sixteen cows were fed for four periods at the New Mexico Station (9). One ration contained 35 pounds of alfalfa and 6 pounds of grain, and the other contained 28 pounds of silage, 22.7 pounds of alfalfa, and 6 pounds of grain. The cows receiving the nonsilage ration produced about 6 per cent more milk but this ration contained about 6 per cent more nutrients.

In the Montana and Pennsylvania trials and especially in the Purdue experiment, considerable credit was given to silage in maintaining or increasing body weight. During the brief periods of some of the experiments gains as great as some of those mentioned could hardly be actual tissue gains but must have been due at least partly to intestinal fill. This tendency of silage to mask the actual body gain by increasing the intestinal fill was pointed out at the Wisconsin Station in 1888 and 1889 (10, p. 36; 15, p. 78). The differences in body weight attributed to fill alone ranged from 16 to 45 pounds. Reference was also made to the effect of silage on body weight by an English writer (1) who in 1888 stated that a silage-fed steer was found to have a dressing out percentage of only 55 in contrast to a nonsilage fed heifer with a dressing out percentage of 71.

#### THE EXPERIMENT

Nine cows were fed alternately a ration of alfalfa hay and grain and a ration of alfalfa hay, grain, and corn silage. For one cow each period of feeding was three calendar months. For all the other cows each period was two calendar months. Each cow was on the experiment from six to ten months.

It was planned that the cows at all times should get 110 per cent of the total digestible nutrients called for by the Savage feeding standard. The hay fed varied from a fairly good grade to a very good grade of alfalfa. The hay and grain ration, therefore, included more digestible protein than the ration containing silage. Two grain mixtures were used. The first five cows,—which were started on the experiment in the spring of 1926,—received a mixture containing 2 parts, by weight, wheat bran; 2 parts yellow corn meal; 1 part linseed-oil meal; and 1 per cent salt. The other four cows,—which were started late in 1926,—were fed a mixture, by weight, of 4 parts corn meal, 3 parts wheat bran, 2 parts soy-bean meal, 1 part linseed-oil meal, and 1 per cent salt. Hay and grain were fed in as nearly as possible equal amounts in all the periods, both being reduced in quantity when silage was fed. The first five cows—four Jerseys and a grade Guernsey—were offered 8 kgm. of silage per day; whereas the

other four cows, which were Holsteins, were given 14 kgm. per day.

The milk was weighed regularly and the butterfat was determined by two-day samples taken the fifth, fifteenth, and twenty-fifth of each month. The feeds were not analyzed, but the total digestible nutrient content of the rations was considered the same as the average figures in the seventeenth edition of "Feeds and Feeding" by Henry and Morrison.

### THE RESULTS

Table 1 gives the data concerning the cows used and the average daily production of milk and butterfat and feed nutrients consumed in each feeding period. The number of periods for each cow varied from three to five. In summarizing the results, when a cow was fed for three periods, the first and third periods were averaged and compared with the second. When a cow was fed for more than three periods, the second and fourth periods were averaged and compared with the third, etc. Whenever one cow was used in more than one comparison, these comparisons were averaged before being averaged for a group.

Table 2 gives the summary of the results. Cows on the non-silage ration produced an average of 2.8 per cent more milk and 4.2 per cent more butterfat than on the silage ration. Only one cow produced more milk on the silage ration. This cow (N201) decreased in milk yield very rapidly during the last month of the last nonsilage period because of her late stage of pregnancy, thus giving to the silage period the advantage found.

In another paper (6) attention was called to the fact that sometimes the comparative decline in production in the different period is a better index of results than a comparison of the period totals. Figure 1 gives the average daily production by ten-day periods for seven of the nine cows, grouped according to the length of time on the experiment and the order of periods. To save space only those cows were used which could be put into groups. Because of advanced pregnancy the third-period declines of cows N201 and N204, and cows N205 and N206 are not shown.

The declines do not show the marked difference between the



TABLE 1

*Detailed information about the cows used, together with the average daily yield of milk and butterfat and the daily consumption of feed nutrients*

COW NUMBER	BREED*	MONTHS ON TEST	AGE	AVERAGE WEIGHT	FEEDING PERIOD	MILK PRODUCTION	BUTTERFAT PRODUCTION	NUTRIENTS RECEIVED	
								Digestible protein	Total digestible nutrients
			years	kgm.		kgm.	kgm.	kgm.	kgm.
600	J	March-April	2½	454	No silage	6.1	0.38	1.37	7.0
600		May-June		465	Silage	5.8	0.38	1.22	7.2
600		July-August		467	No silage	5.2	0.34	1.33	6.9
600		September-October		469	Silage	4.4	0.30	0.90	5.5
600		November-December		478	No silage	3.3	0.24	1.13	5.9
605	J	March-April	2½	388	No silage	7.5	0.38	1.36	7.0
605		May-June		407	Silage	6.9	0.40	1.24	7.0
605		July-August		400	No silage	6.7	0.37	1.26	6.5
605		September-October		423	Silage	5.2	0.32	1.13	6.5
458	J	April-May	4½	411	Silage	10.1	0.62	1.36	7.3
458		June-July		402	No silage	9.5	0.60	1.46	7.5
458		August-September		404	Silage	8.5	0.55	1.25	7.3
458		October-November		412	No silage	7.5	0.51	1.21	6.2
499	J	January-February	2½	401	No silage	7.3	0.47	1.35	7.0
499		March-April		415	Silage	7.4	0.46	1.22	7.1
499		May-June		420	No silage	7.8	0.49	1.27	6.6
499		July-August		416	Silage	7.2	0.44	1.16	6.5
99	GG	April-May-June	5½	429	No silage	10.9	0.42	1.33	6.9
99		July-August-September		420	Silage	8.5	0.34	1.18	6.8
99		October-November-December		466	No silage	6.7	0.27	1.38	7.1
N201	H	November-December	7+	606	No silage	16.2	0.57	2.19	10.1
N201		January-February		624	Silage	15.1	0.54	1.81	9.8
N201		March-April		632	No silage	10.4	0.38	2.00	9.2
N204	H	November-December	5+	587	No silage	10.2	0.36	1.81	8.3
N204		January-February		613	Silage	8.3	0.28	1.29	7.7
N204		March-April		607	No silage	7.4	0.25	1.60	7.4
N205	H	December-January	5+	493	Silage	19.2	0.72	2.11	11.4
N205		February-March		502	No silage	20.2	0.75	2.56	11.8
N205		April-May		519	Silage	17.3	0.66	1.87	10.1
N206	H	December-January	5+	593	Silage	19.5	0.67	2.19	11.6
N206		February-March		599	No silage	18.3	0.70	2.53	11.6
N206		April-May		633	Silage	14.8	0.56	1.93	10.5

\* J, Jersey; GG, Grade Guernsey; H, Holstein.

silage and nonsilage rations that was found in the experiments carried out at Purdue (8). On the contrary, the production curves confirm the figures in table 2 in showing that there was

TABLE 2

*Summary of results*

The figures represent average daily milk yields, etc.

	COW NUMBER	AVER- AGE MILK YIELD	AVER- AGE BUTTER- FAT YIELD	NUTRIENTS REQUIRED		NUTRIENTS FED		PLANE OF NUTRI- TION*
				Digesti- ble protein	Total digesti- ble nutri- ents	Digesti- ble protein	Total digesti- ble nutri- ents	
		<i>kgm.</i>	<i>kgm.</i>	<i>kgm.</i>	<i>kgm.</i>	<i>kgm.</i>	<i>kgm.</i>	<i>per cent</i>
No silage ...	600	5.1	0.33	0.76	6.2	1.30	6.8	109.7
	605	6.9	0.38	0.76	6.3	1.29	6.7	106.3
	458	9.0	0.58	1.06	7.7	1.40	7.2	93.5
	499	7.7	0.49	0.94	7.1	1.29	6.7	94.4
	99	8.8	0.35	0.89	6.7	1.36	7.0	104.5
	N201	13.3	0.48	1.21	8.7	2.10	9.7	111.5
	N204	8.8	0.31	0.92	7.1	1.71	7.9	111.3
	N205	20.2	0.75	1.60	10.5	2.56	11.8	112.4
	N206	18.3	0.70	1.55	10.5	2.53	11.6	110.5
	Average	10.9	0.49	1.08	7.9	1.73	8.4	106.3
Silage.....	600	5.1	0.34	0.77	6.2	1.06	6.4	103.2
	605	6.5	0.38	0.82	6.3	1.22	6.9	109.5
	458	8.9	0.57	1.05	7.6	1.28	7.3	96.1
	499	7.4	0.46	0.91	6.9	1.21	7.0	101.4
	99	8.5	0.34	0.85	6.4	1.18	6.8	106.3
	N201	15.1	0.54	1.32	9.3	1.81	9.8	105.4
	N204	8.3	0.28	0.89	7.0	1.29	7.7	111.6
	N205	18.3	0.69	1.50	10.0	1.99	10.8	108.0
	N206	17.2	0.62	1.44	10.0	2.06	11.1	111.0
	Average	10.6	0.47	1.06	7.7	1.46	8.2	106.5

\* Plane of nutrition is the total digestible nutrients eaten by the cow expressed in percentage of the requirement of the Savage standard.

no appreciable difference in the two rations used. Under the conditions of the experiment, therefore, silage was not effective in increasing milk production.

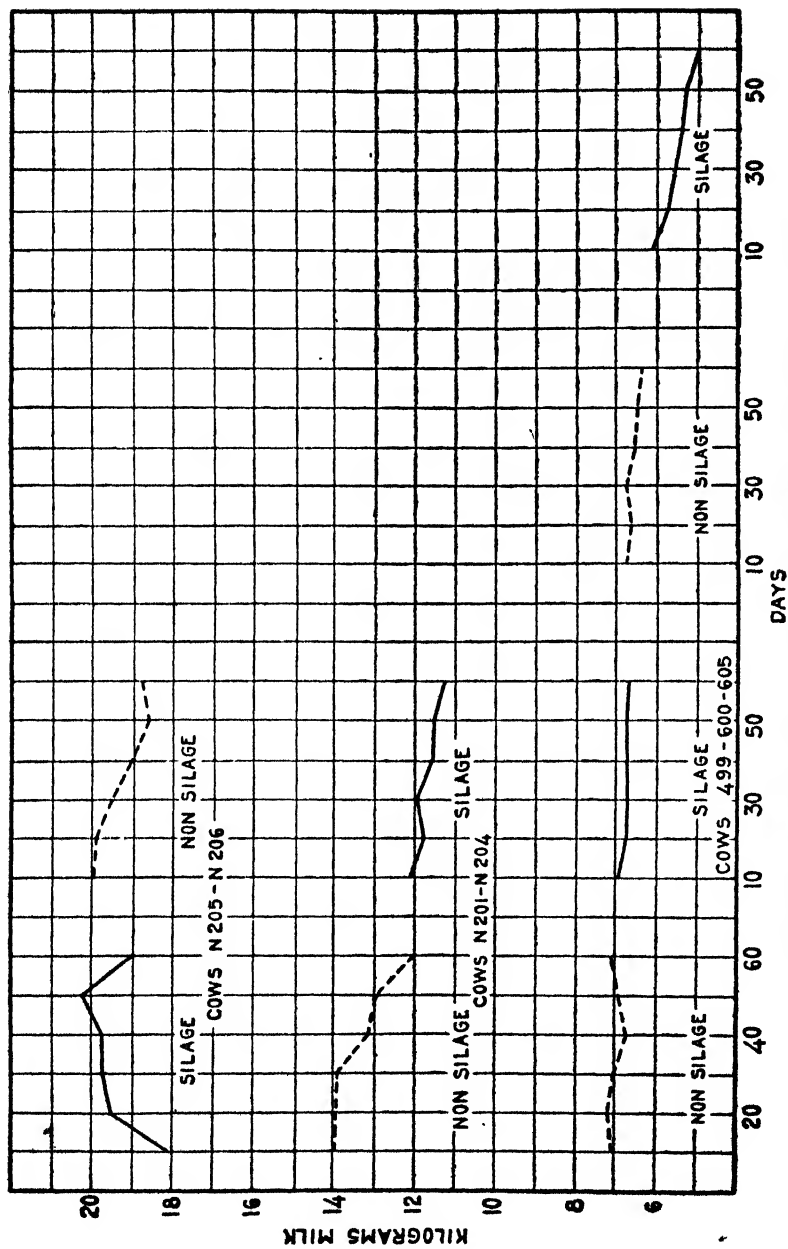


FIG. 1. DECLINE IN MILK YIELD DURING PERIODS WITH AND WITHOUT SILAGE

The cows were supposed to consume 110 per cent of the total digestible nutrients called for by the Savage feeding standard. Although five of the cows did not consume this quantity of feed, yet the nutrients consumed under the two conditions of the study were quite comparable. The figures indicate that these five cows consumed on the average exactly the same quantity of nutrients whether silage was included in the ration or not. In the case of two or three of these cows, however, the addition of silage did seem to increase very slightly the quantity of nutrients they would consume.

### CONCLUSIONS

Under the conditions of this experiment, whether silage was fed or not made practically no difference in the milk and butter-fat yields. It should be pointed out, however, that in the experiment very moderate quantities of silage were added to a good ration, which, even after this addition, still contained a liberal quantity of good legume hay. The results throw no light on the question as to what would happen if, for instance, the hay were largely or entirely replaced by silage. Furthermore, no attempt was made in the study to disprove the fact that silage is a very economical and useful feed in the practical dairy ration or that an otherwise poor ration may be enhanced in value by the addition of silage.

From the results of the experiments, however, it seems that the factor of silage succulence does not increase the value of a ration containing an ample quantity of good alfalfa hay and a satisfactory grain mixture. Thus for experimental purposes, it seems justifiable to simplify the basal ration by leaving out the silage.

### REFERENCES

- (1) A correspondent (T. C. S.). Silage versus live weight of cattle. London (Eng.) Agr. Gazette, February 20, 1888, p. 167.
- (2) BARTLETT, J. M., AND MERRILL, L. H.: Maine Agr. Exp. Station Report, 1889, p. 69.
- (3) BECHDEL, S. I.: Penn State Exp. Sta. Bulletin 178 (1923).
- (4) CARROLL, W. E.: Utah State Exp. Sta. Bulletin 190 (1924).
- (5) CLARK, R. W.: Montana State Exp. Sta. Bulletin 94 (1913).

- (6) CONVERSE, H. T.: The effect on milk production of feeding more than the Haecker, Eckles, and Savage requirements. Jour. Dairy Science, vol. 9, no. 4 (1926).
- (7) COOKE, W. W., AND HILLS, J. L.: Vermont State Exp. Sta. Reports, 1891, p. 75, and 1892, p. 163.
- (8) FAIRCHILD, L. H., AND WILBUR, J. W.: Purdue University Exp. Sta. Bulletin 297 (1925).
- (9) FOSTER, LUTHER, AND MEEKS, J. R.: New Mexico State Exp. Sta. Bulletin 122 (1920).
- (10) HENRY, W. A.: Wisconsin State Exp. Sta. Report, 1888, p. 10.
- (11) HILLS, J. L.: Vermont State Exp. Sta. Report, 1900-1901, p. 350.
- (12) SNYDER, W. P.: Superintendent North Platte Station, Nebraska State Exp. Sta. Report, 1923, p. 34.
- (13) WILLIAMS, C. G.: Ohio State Exp. Sta. Bulletin 155 (1904).
- (14) WILLIAMS, R. H., AND CUNNINGHAM, W. S.: Arizona State Exp. Sta. Report, 1917, p. 468.
- (15) WOLL, F. W.: Wisconsin State Exp. Sta. Reports, 1889, p. 71, 1890, p. 80, and 1891, p. 49.

# PHYSICAL FACTORS INFLUENCING THE FORMATION AND FAT CONTENT OF GRAVITY CREAM\*

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## INTRODUCTION

The rate of creaming of milk, the depth of the cream layer, and the fat content of the cream and skim milk in gravity creaming have been the subjects for a large number of investigations. It has been shown that various treatments markedly affect the creaming of milk and since many of these treatments also produced other accompanying changes in the milk the conclusion, frequently, has been drawn that a causal relation existed between the creaming of milk and some of the accompanying phenomenon, when actually this was not the case.

Arnold (1) believed that as the milk cooled rapidly the fat did not cool as fast as the plasma, and that therefore cooling produced a great difference in density and caused the cream to rise.

Babcock (2) was the first to call attention to the clumped condition of the fat globules in normal raw milk but he then concluded that clumping tended to prevent cream from rising. Woll, Babcock and Russell (40) and Hammer (15) however associated the clumping of fat globules with creaming. The clumping of fat globules as it influences creaming was specifically studied by Rahn (28) van Dam and Sirks (33) Hekma and Sirks (19) Sirks (31) Brouwer (6) and Hekma (18) who came to the conclusion that normal gravity cream was obtained only when the fat globules were clumped. On the other hand Palmer and Anderson (26) and Palmer, Hening and Anderson (27) have presented the results of experiments which they interpret as

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evidence against the major importance of the clumping of the fat globules as an explanation of creaming. They, however, offer no alternate explanation of the mechanism of creaming. Fleischmann (14) and Bancroft (5) have expressed the view that the larger globules overtake the smaller globules and that they then rise together exerting as Bancroft expresses it, a filtering action.

A study of the gravity creaming of milk has led us to the conclusion that the clumping of the fat globules plays the major rôle in explaining the creaming phenomenon. Some of our evidence for this conclusion is presented in this paper.

#### RATE OF RISE OF SINGLE FAT GLOBULES

Equations for the rise of fat in milk have been given by Fleischmann (14) and Richmond (29). Both of their equations contain arbitrary constants. In 1847 Stokes developed the equation for the rate of movement of small spheres at uniform velocity in a viscous medium. Since this equation has been found to hold for so many systems, it should be applicable for calculating the rate of rise of fat globules in milk. Svedberg and Estrup (32) used Stokes equation to determine the size and distribution of fat globules in milk, but some error in their calculations leads to abnormally large sizes for the fat globules. van der Burg (35) called attention to the fact that the individual globules of milk should rise according to Stokes law and Rahn (28) and van Dam and Sirks (33) have presented experimental evidence to show that they actually do.

Stokes equation is as follows:

$$V = \frac{2 r^2 (d_s - d_f) g}{9 \eta} \quad (1)$$

Where  $V$  is the velocity of rise in centimeters per second,  $r$  is the radius of the fat globule,  $d_s$  and  $d_f$  are the density of the skim milk and the fat respectively,  $g$  is the gravitational constant 980 dynes, and  $\eta$  is the viscosity of the skim milk.

The way in which the temperature affects the rate of rise of the individual fat globules of various sizes can be best appreciated by giving the results of calculations using Stokes equation. In

order to calculate the rate of rise of the fat globules it is necessary to know the density of the fat and the skim milk and the viscosity of the skim milk. The viscosity, expressed in poises, and density of the skim milk as taken from the work of Whitaker, Sherman, and Sharp (37) for four temperatures are given in table 1, together with the density of solid milk fat as determined at the same temperatures.

Table 1, indicates that according to Stokes law the individual fat globules should rise faster at the warmer temperatures for two reasons, first, because of the decrease in viscosity, and second, because the action of the force of gravity is greater due to the greater spread between the density of the fat and the skim milk. In addition the fat globules would be slightly larger at the higher

TABLE 1

*Density of skim milk and milk fat and viscosity of skim milk at various temperatures*

	TEMPERATURE			
	5°C.	15°C.	24°C.	25°C.
Density of skim milk.....	1.0365	1.0348	1.0324	1.0322
Density of fat.....	0.9612	0.9421	0.9227	0.9208
Difference.....	0.0753	0.0927	0.1097	0.1114
Viscosity of skim milk .....	0.0296	0.0210	0.0158	0.0154

temperatures due to expansion of the fat. The way in which the constants of the milk affect the rate of rise of the fat globules of different sizes can be understood best by calculating the rate of rise at different temperatures. The results of such calculations are given in table 2.

If we consider that the average diameter of the fat globules in milk is  $4\mu$ , it would take 276 hours for the average fat globule to rise from the bottom to the top of a quart milk bottle at 5°C., and 97 hours for it to rise at 25°C. These calculations indicate that faster creaming would occur at 25°C. than at 5°C. while common experience indicates that creaming should be carried out at near the lower temperature. These calculations show that the fat in gravity creaming does not rise as individual globules,



provided that Stokes law holds for the rise of the individual fat globules of milk.

Rahn (28) measured the rate of rise microscopically by two methods. First, by following the rise in a chamber on a slide by keeping the globule in focus and reading the vernier on the microscope to determine the distance traversed by the fat globule, and second, measuring the rate of rise in a slide similar to a blood counting chamber with the stage in a vertical and the tube of the

TABLE 2

*Rate of rise at various temperatures of individual fat globules, and the time required for such globules to rise from the bottom to the top of a quart milk bottle (220 mm.), as calculated by Stokes' equation*

DIAMETER OF FAT GLOBULES	TEMPERATURE					
	5°C.		15°C.		25°C.	
	Rate of rise per hour	Time to cream in milk bottle	Rate of rise per hour	Time to cream in milk bottle	Rate of rise per hour	Time to cream in milk bottle
#	mm.	hours	mm.	hours	mm.	hours
1	0.0498	4,417	0.0881	2,497	0.142	1,550
2	0.1994	1,103	0.352	625	0.567	388
3	0.449	490	0.792	278	1.28	172
4	0.798	276	1.409	156	2.27	97
5	1.25	176.	2.201	100	3.54	62
6	1.79	123	3.17	69	5.10	43
8	3.19	69	5.64	39	9.07	24
10	4.99	44	8.81	25	14.2	15.5
14	9.77	22.5	17.26	12.7	27.8	8.0
16	12.76	17.2	22.54	9.8	36.3	6.1
20	19.94	11.0	35.22	6.2	36.7	3.9

microscope in a horizontal position. The time was determined with a stop watch. The results which he obtained by these two methods were in essential agreement, except that the larger fat globules rose more slowly by the second method than they did by the first. The walls of the chamber used in his second method were so close together that they probably slowed down the movement of the fat globules. This effect would naturally become more pronounced the larger the fat globules. van Dam

and Sirks (33) measured the rate of rise of a number of fat globules  $3.3\mu$  in diameter finding 1.8 mm. per hour. They calculated the rate according to Stokes equation to be 1.4 mm. per hour.

We have determined how accurately the calculated rate of rise by Stokes' law agrees with the experimentally determined rate. Since temperature so markedly affects the density and viscosity values, it is necessary to make the experimental determinations on the rate of rise at a known temperature. An insulated room was available which did not change rapidly in temperature. Therefore the experimental rate of rise was determined at the temperature of this room. The average temperature was near  $24^{\circ}\text{C}.$ ; the deviation was usually less than one-half although occasionally it was as much as  $1\frac{1}{2}$  degrees either above or below this temperature.

A wide microscopic slide was used which had three trenches cut lengthwise in the slide. These trenches were 5 mm. wide, 0.5 mm. deep and 70 mm. long. A cover glass large enough to cover all three trenches was sealed to the slide with an appropriate mixture of paraffin and paraffin oil. Samples of skim milk or skim milk to which a drop of whole milk had been added were drawn into the trenches and the ends were sealed by means of paraffin. The microscope was tilted and the slide mounted in a perpendicular position on a mechanical stage. The rate of rise was measured by means of a stop watch by timing the passage of the globules through distances as indicated on an eye piece micrometer. A globule rising in the center of the chamber was selected for measurement in order to minimize the retarding effect of the walls. The size of the globules was estimated at the same time. It was not possible to determine the size of the globules with any considerable accuracy, because they were moving, and the best that could be done was to estimate their size to within a few tenths of a  $\mu$ . Fat globules of the large sizes were obtained by mixing heated cream with skim milk. A great deal of difficulty was encountered with convection currents. It was necessary to place the adjusted microscope and preparation in a box with only the eye piece protruding. This box was kept in the insulated room and the preparation was given several

TABLE 3

*Rate of rise of individual fat globules in milk at 24°C., as calculated by Stokes' equation and as determined experimentally*

DIAMETER OF FAT GLOBULES	RATE OF RISE PER HOUR		AVERAGE DEVIATION FROM THE MEAN EXPERIMENTAL VALUE	NUMBER OF OBSERVATIONS
	Calculated	Experimental average		
$\mu$	mm.	mm.		
1.8	0.44	0.55	0.03	6
2.0	0.54	0.67		1
2.25	0.70	0.85	0.15	19
2.40	0.78	0.97	0.39	4
2.52	0.86	1.05	0.07	2
2.70	0.99	1.13	0.15	34
3.20	1.38	1.26	0.17	16
3.60	1.76	2.04	0.80	21
4.50	2.74	2.70	0.33	8
4.95	3.32	3.52	0.28	2
5.40	4.0	3.9	0.6	10
5.75	4.5	5.1		1
6.30	5.3	4.4	1.0	5
6.8	6.3	5.5	1.1	3
7.2	7.0	6.6	1.4	23
7.56	7.8	8.3		1
8.2	9.1	9.7	1.0	5
8.5	9.8	11.3	0.7	3
9.9	13.3	14.7		1
10.8	15.9	19.3	1.9	18
12.6	21.6	27.6	3.1	14
14.4	28.3	29.8	1.9	11
16.2	35.8	38.8	2.9	5
18.0	44.1	41.1	5.0	6
19.8	53.3	49.7	0.7	2
21.6	63.7	66.0	11.4	3
25.2	85.8	85		1
27.0	99.5	124	5.0	4
30.6	127	119		1
32.4	143	161		1
34.2	160	143		1
36	177	174	20	3
37.8	195	185		1
39.6	214	215		1
41	229	242	29.0	2

hours time to come to temperature equilibrium. The artificial light used was passed through water. The increase in temperature caused by the light and by the presence of the observer in the room would start convection currents in the preparation in less than an hour, so that no more readings were taken when convection currents were noted. These convection currents could be recognized by the behavior of the smallest globules.

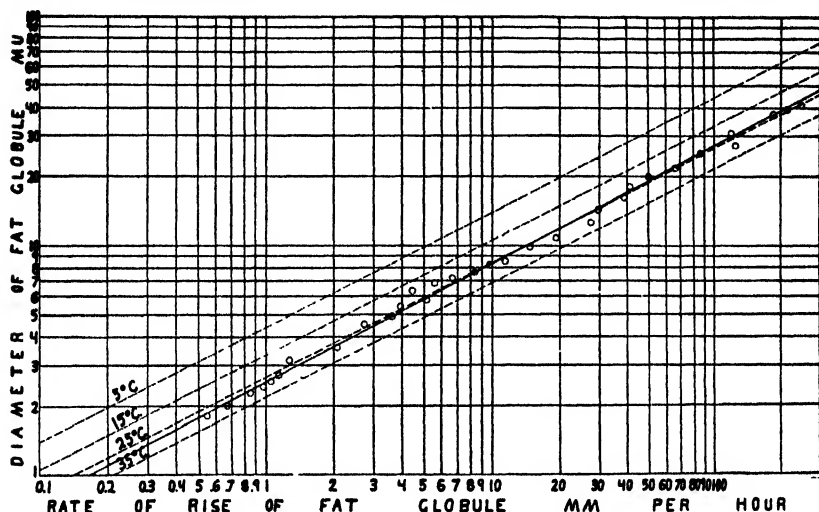


FIG. 1. LOGARITHMIC PLOTTING OF THE RATE OF RISE OF INDIVIDUAL FAT GLOBULES OF VARIOUS SIZES

The broken lines express the rate of rise as calculated from Stokes equation for the different temperatures. The circles represent the experimental values obtained at 24°C. The solid line represents the best line which can be drawn through the experimental points.

Table 3 contains the experimental data obtained on the rate of rise of individual fat globules in skim milk. This table includes only the data obtained after the technique was perfected. When the logarithms of the rate of rise, as calculated by Stokes' equation, are plotted against the logarithms of the diameter of the fat globules, the results fall on a straight line. The calculated rates of rise at 5°, 15°, 25°, and 35°C. are represented in figure 1 by the broken lines and the experimental data of table 3 by the

circles. The agreement between the calculated and experimental rates of rise is excellent considering the experimental difficulties involved.

Since the logarithmic plotting of this data approximates a straight line, the equation for a straight line was fitted to the data.

$$\log y = A + B \log x \quad (2)$$

and the constants  $A$  and  $B$  were calculated by the method of least squares. The equation for the best line which can be drawn to represent the experimental data as given in table 3 is

$$\log y = 0.4059 + (0.5140) \log x \quad (3)$$

where  $y$  is the diameter of the fat globules in  $\mu$ , and  $x$  is their rate of rise in millimeters per hour. The constant  $B$  represents the tangent of the angle or the slope of the line, and should be 0.5000 since the velocity is proportional to the square of the radius.

In calculating the rate of rise from Stokes' equation, slight errors may be made in the densities used, in the viscosity, or in the temperature. These will all affect the constant  $A$  of equation (2), but will have no influence upon the constant  $B$  or the slope. The experimental slope as given by equation (3) agrees with the slope given by Stokes' equation within 2.8 per cent. The solid line in figure 1 was plotted from equation (3).

In determining the rate of rise experimentally the greatest errors occurred in measuring the rate of rise and size of the smallest fat globules. Convection currents produced proportionately, a greater effect on the rising of the small fat globules than on the large ones. The movement of the smallest globules was so slow that there was probably a tendency to select the globules which were rising aided by very slight convection currents. This error would tend to increase the slope of the line. An error of  $0.2\mu$  in estimating the size of the smallest globules would entirely account for the difference between the experimental and calculated rate of rise.

If the first five points in table 3 and figure 1 are omitted, and the linear equation is calculated for the remaining 30 points by the method of least squares, the following result is obtained:

$$\log y = 0.4273 + (0.4995) \log x \quad (4)$$

The slope of the line in equation (4) is within 0.1 per cent of the slope calculated by Stokes' equation.

In order to show more clearly the relation between the experimental and calculated rates of rise, table 4 was constructed. The rates of rise at 24° and 25°C. were calculated using Stokes' equation; also the experimental rates of rise were calculated by equations (3) and (4). It will be noted that the rates of rise given by equation (4) fall between the calculated rates for 24° and 25°C. Since 1°C. makes a difference of about 4 per cent in the rate of rise, the agreement between the calculated and experimental rates of rise is exceptionally good. This good agreement is

TABLE 4

*Rate of rise of fat globules in millimeters per hour, as calculated by Stokes' equation and as determined experimentally*

SIZE OF FAT GLOBULE	CALCULATED BY STOKES' EQUATION		EXPERIMENTAL CALCULATED FROM	
	24°C.	25°C.	Equation (4)	Equation (3)
<i>n</i>				
1	0.136	0.142	0.140	0.163
2	0.544	0.567	0.560	0.627
4	2.18	2.27	2.24	2.41
6	4.90	5.10	5.04	5.31
10	13.6	14.2	14.02	14.4

probably better than the experimental errors justify and was obtained only because of the large number of determinations made by very careful experimentation, and only after a number of experimental difficulties were overcome. It may be concluded that Stokes' equation expresses quite accurately the rate of rise of individual fat globules in milk, when the globules are far enough apart so they exert no effect on each other. Rahn (28) measured the rate of rise of individual fat globules in whole milk. Since his results are in essential agreement with those reported here, it is probable that the individual fat globules in whole milk exert no pronounced influence upon the rate of rise of each other.

## RATE OF RISE OF FAT CLUMPS

Since the individual fat globules rise too slowly to account for gravity creaming some other explanation must be sought. Rahn (28), van Dam and Sirks (33) and van der Burg (36) have considered that clumping of the fat globules was necessary in order that the gravity cream might rise in a reasonable time. It has long been known that the fat globules in normal raw milk are present mainly in the form of clumps or clusters, and not as separate fat globules. These clusters should rise much faster than the individual globules, because according to Stokes' equation the rate of rise depends on the square of the radius; thus the rate increases very rapidly with size. On the other hand, the clusters would contain plasma in the spaces between the individual globules, and consequently the rate of rise of a cluster would not be as rapid as that of a fat globule of equal size. It is possible for uniform spheres under the condition of maximum packing to occupy 74.04 per cent of a volume without deformation. This condition of packing is independent of the diameter of the spheres. It is not known exactly what part of the volume of a cluster is occupied by fat and what part by skim milk. The ratio of volume of fat to volume of plasma in the cluster varies with the conditions. Assuming that the clusters are spherical, calculations using Stokes' equation were made on the basis of clusters containing 75, 50, and 25 per cent fat. The results are presented in table 5, and give some idea of the effect of the various factors on the rate of rise of clusters.

It will be observed that some of the calculated rates of rise as given in table 5, are fast enough to account for normal creaming, provided clusters of sufficient size are found in milk. The size and rates of rise of a number of clusters found in milk were measured. The samples used for these determinations were prepared by adding a drop or two of cream or whole milk to skim milk. The clusters were of various shapes and sizes. Some of the clusters were apparently spherical, the fat globules being closely packed in the cluster, while others were of irregular shape with projections and frequently they had holes in them. After

TABLE 5

*Rate of rise of fat clusters at various temperatures, and the time required for such clusters to rise from the bottom to the top of a quart milk bottle (220 mm.), as calculated by Stokes' equation, assuming that the clusters are spherical and that the fat occupies 25, 50, and 75 per cent of the volume of the cluster*

DIAMETER OF CLUSTERS	75 PER CENT FAT		50 PER CENT FAT		25 PER CENT FAT	
	Rise per hour	Time to cream in milk bottle	Rise per hour	Time to cream in milk bottle	Rise per hour	Time to cream in milk bottle

5°C.

$\mu$	mm.	hours	mm.	hours	mm.	hours
10	3.75	58.7	2.50	88.0	1.25	176.0
20	15.0	14.7	9.98	22.0	4.99	44.0
30	33.7	6.5	22.44	9.8	11.22	19.6
40	59.8	3.68	39.8	5.5	19.9	11.0
60	135	1.63	89.8	2.5	44.9	4.9
80	239	0.92	159.6	1.38	79.8	2.76
100	375	0.59	250	0.88	125	1.76
150	841	0.26	560	0.39	280	0.78
200	1,500	0.15	998	0.22	499	0.44

15°C.

10	6.49	33.9	4.31	51.0	2.16	102
20	25.9	8.5	17.2	12.8	8.6	25.6
30	58.4	3.8	39.0	5.65	19.5	11.3
40	104	2.12	69.2	3.17	34.6	6.34
60	234	0.94	155	1.42	77.5	2.84
80	415	0.53	277	0.79	139	1.58
100	649	0.34	431	0.51	216	1.02
150	1,360	0.16	973	0.23	487	0.45
200	2,590	0.09	1,720	0.13	860	0.26

25°C.

10	10.6	21.0	7.09	30.9	3.55	61.9
20	42.5	5.25	28.4	7.75	14.2	15.5
30	95.7	2.33	63.8	3.45	31.9	6.90
40	170	1.31	113.4	1.94	56.7	3.88
60	383	0.581	255	0.86	128	1.72
80	681	0.328	454	0.49	227	0.969
100	1,060	0.210	709	0.31	355	0.620
150	2,390	0.093	1,600	0.14	800	0.275
200	4,250	0.052	2,840	0.08	1,420	0.155



considerable difficulty a technique was developed for measuring the rate of rise of the clusters. All of these determinations were made in trenches 5 mm. wide, 1 mm. deep and 70 mm. long. Here again care was taken to avoid as much as possible the error caused by convection currents. Both the horizontal and vertical diam-

TABLE 6  
*Rate of rise of fat clusters in milk at 25°C.*

DIAMETER OF CLUSTER	AVERAGE RATE OF RISE PER HOUR	MEAN DEVIATION	NUMBER OF OBSER- VATIONS	DIAMETER OF CLUSTER	AVERAGE RATE OF RISE PER HOUR	MEAN DEVIATION	NUMBER OF OBSER- VATIONS
$\mu$	mm.			$\mu$	mm.		
9-10	8.0	0.1	3	75-80	323	11	11
10-11	9.9	0.6	2	80-85	327	102	3
11-12	11.5	2.7	4	85-90	419	102	9
13-14	14.9	3.1	5	90-95	462	104	2
14-15	15.9	1.6	4	95-100	665	128	11
15-16	17.6	0.9	2	100-110	720	240	2
16-17	19.3	2.6	6	110-120	791	152	9
17-18	28.1	13.4	3	120-130	884	303	5
19-20	22.3	2.0	4	130-140	1,238	202	5
20-22	26.0	4.3	3	140-150	981	171	8
22-24	36.0	11.4	5	150-160	1,331	145	7
24-26	30.1	3.6	4	170-180	1,255	200	7
26-28	49.7	17.9	11	190-200	1,302	198	3
28-30	35.5	2.6	9	200-220	1,228	385	6
30-32	64.0	20	9	220-240	2,082	317	6
34-36	102	22	13	240-260	2,880	0	2
38-40	141	15	14	260-280	1,847	210	2
42.5-45	133	27	7	280-300	1,893	658	3
47.5-50	155	33	8	300-320	1,454	146	2
50-52.5	208	15	4	320-340	3,200	1,600	2
55-57.5	211	23	6	380-400	2,445	289	3
57.5-60	241	60	8	425-450	5,200	1,333	3
60-65	263	46	16	475-500	3,840	960	2
65-70	265	28	4	700-750	5,800	2,000	2
70-75	341	100	17	750-800	5,600	1,067	3

eters of the clumps were measured, and the average taken as the true diameter. A measurement of the other important horizontal diameter is impossible, so that the average diameter cannot be determined accurately, but an average of the two diameters which were measured probably approaches the true diameter. A

microphotograph of an example of typical clumps in milk is given in plate 1 A. A clump similar to those selected for measuring the rate of rise is reproduced in plate 1 B. While a number of clumps of all kinds were measured, only the data for those clumps which appeared to be compact and spherical are given in table 6. The large clumps would move across the field so rapidly that no accurate measurement of size could be made while their velocity

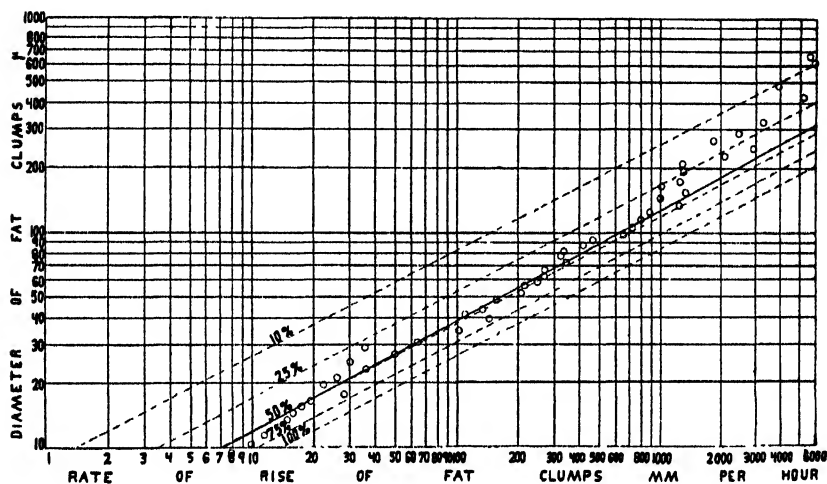


FIG. 2. LOGARITHMIC PLOTTING OF THE RATE OF RISE OF CLUSTERS OF FAT GLOBULES OF VARIOUS SIZES AT 25°C.

The broken lines express the rate of rise as calculated from Stokes equation for clusters of different fat content. The circles represent the experimental values obtained at 25°C. The solid line represents the best line which can be drawn through the experimental points.

was being measured. The fat clusters were then followed by the aid of the mechanical stage, and the measurements of size made as accurately as possible while the fat cluster and mechanical stage were moving in opposite directions. By this method the cluster could be held in view.

The data of table 6 are expressed graphically in figure 2. The broken lines indicate the rate of rise calculated from Stokes' equation for clusters containing various amounts of fat. The

experimental data are represented by the circles, while the solid line is given by the following equation:

$$\log y = 0.552 + (0.519) \log x \quad (5)$$

It was calculated from the experimental data by the method of least squares for clusters between 13 and  $140\mu$  in size. In this equation  $y$  indicates the diameter of the cluster in  $\mu$  and  $x$  the rate of rise in millimeters per hour. The walls of the chamber in which the clusters rose slowed down the rate of clusters larger than about  $140\mu$  so their rates of rise were not used in calculating equation (5). The slope of the line in equation (5) agrees with the slope indicated by Stokes' equation within 3.8 per cent. This good agreement indicates that the rate of rise is proportional to the square of the radius. The experimental error in determining the rate of rise of clusters was great due to irregularities in shape, to convection currents set up by the movement of clusters other than the one being measured, and to variations in packing of the fat globules in the clusters.

The experimental rates of rise of fat clusters indicates that they contained slightly less than 50 per cent of fat. As only the compact clusters were selected for these measurements, 50 per cent is probably near the upper limit for the fat content of the normal clusters. During these experiments a considerable proportion of clusters were observed which were not compact, and such clusters always rose at a much slower rate. Also many clusters of irregular shape were observed as shown in plate 1 A.

These experiments show that if the fat globules rise as individuals, creaming by the gravity process will not take place in a time short enough to account for normal gravity creaming, but if the fat globules are present in clusters of sufficient size, the normal creaming time is easily explained. This conclusion is in agreement with the previously expressed views of Rahn (28) and van Dam and Sirks (33). That the clusters in milk are of sufficient size to rise rapidly is clearly shown by plate 1 A which is a microphotograph of the clusters in milk which had been pasteurized for thirty minutes at  $63^{\circ}\text{C}$ . ( $145^{\circ}\text{F}$ .) and then cooled. This milk had not creamed at the time the sample was taken but it creamed

shortly afterward. This microphotograph shows clearly that milk pasteurized in the holding process at not too high a temperature, if properly cooled and allowed to remain undisturbed will again form clusters very similar to those in properly treated raw milk.

The literature contains the reports of several attempts to correlate the clumping of the fat globules with gravity creaming. Babcock (2) at first, at least, believed that clumping retarded gravity creaming, Palmer, Hening, and Anderson (27) say of some of their experiments: "These results are wholly irreconcilable with those theories which attempt to explain creaming variations by means of fat globule clustering or its absence." On the other hand it is evident that Woll, Babcock and Russell (40) associated the normal creaming of milk with the presence of clusters and its failure to cream as due to the absence of clusters. They did not however, offer any explanation for this behavior or enlarge upon it in any way. Hammer (15) also called attention to the possible variation in the clumps as explaining the variation in the creaming of milk. Rahn (28) van Dam and Sirks (33) Hekma and Sirks (19) Sirks (31) Brouwer (6) Hekma (18) and van der Burg (36) were inclined to believe that clumping of the fat globules was the controlling factor in gravity creaming.

Perhaps some investigators have failed to recognize the important relation between clumping and creaming due to faulty methods used in determining the clumping. A survey of the microphotographs of clumps of fat globules as previously published makes this clear for most of these photographs do not show the real large clusters that are necessary for quick forming deep cream layers but show only what are probably fragments of the large clusters which were originally present. It is only occasionally that the milk can be diluted to any considerable extent without breaking up the large clusters. Occasionally the slightest shearing force will destroy the clusters. In the case of such samples we mounted the unclustered milk in a long trench on a microscopic slide and allowed the clustering to take place in the perpendicular trench; preferably on the stage of a microscope. A magnification of about 100 times seems to be well

suitd to the observation of clumps. If too high a magnification were used a portion of a clump would frequently occupy the entire field. A layer of milk about 0.2 to 0.5 mm. deep, 5 mm. wide and 70 mm. long seemed to be suitable for the observation of the clusters.

Our experience indicates that the most successful way to study the relation between clustering and creaming is to set up a microscopic creaming cell at the same time that a bulk sample of the milk is placed in a creaming cylinder. Both microscope and creaming cylinder should be placed at the same temperature and both observed from time to time.

The creaming time elapsing after cooling warm milk can be divided into two parts. First, the time necessary for the fat globules to clump, and second, the time required for the clumps to rise after they have formed. A sharp distinction between these two parts of the creaming time cannot be drawn since the clumps grow in size for some time. The clumping time is greatly influenced by temperature. When milk properly pasteurized by the holding method is cooled rapidly to a low temperature, the clumping usually takes place in from five to thirty minutes. The length of time required for the clumps to rise after they have formed depends largely on their size, shape, and compactness.

In some instances where milk at room temperature was made tenth-normal with sodium hydroxide (10 cc. of normal NaOH to 90 cc. of milk) the clumps took several hours to form but less than an hour to rise. However, when the alkali was added to the milk at a temperature of about 45°C. and was set to cream at 35°C., the clumping and creaming usually occurred within thirty minutes. When the creaming was carried out in a room at 0°C. the cream layer formed was very deep, often occupying 60 per cent or more of the volume. The depth of this layer decreased with time. The clumps were very large, some of them being a centimeter or more in diameter.

Clausnizer (9) observed that if alkali was added to milk (probably cooled) and the milk was set to cream at a cold temperature, creaming did not occur, but if the milk was warmed to a higher temperature a cream layer was obtained.

Some of our observations indicated that when cold milk was made tenth-normal with respect to sodium hydroxide, and was then creamed at 0°C., the cream occupied about 95 per cent of the volume. In fact the cream layer at first was overlooked in some experiments, because it was so near the bottom of the creaming cylinder. Microscopic observations indicated that the fat globules were clumped. After the cream layer had formed at the low temperature it contracted markedly on warming to a temperature of 25°C. or above.

Henseval (20) and Marcas (25) observed that milk which did not cream at low temperatures did cream if the milk was heated to 40° to 60°C. It is possible that the fat was in such a clumped condition that the cream layer occupied almost the entire volume of the milk and was therefore overlooked. Upon warming such milk the customary shrinkage in the depth of the cream layer probably occurred and the resulting cream layer was then recognized.

The clumps formed in milk may vary greatly in size, some clusters may contain only a few globules while others will contain thousands, and under some conditions, are large enough to be seen with the naked eye through the sides of a milk bottle. Clusters vary considerably in their ability to resist being broken up by agitation; the weak clusters will be broken up by a relatively slight jar of the microscope, while the firmest will break down only slowly as the milk is stirred. The clusters are more firm at the lower temperatures. The weakening of the clusters due to an increase in temperature was observed by preparing the microscopic creaming slide in a cold room and then bringing it into a warm room. It was possible, however, to work with some samples of milk at room temperature without very materially weakening the clusters but on the whole very much better clustering was observed and followed at lower temperatures. This is especially true in studying the clusters in the cream layer. The microphotographs were taken in a room with a temperature of about 7°C. (45°F.).

The effect of temperature on clustering was observed in another connection. Babcock (2) made the statement that freshly drawn

milk was not clustered but that it clustered within a few minutes after milking. We have confirmed his observation and have observed in addition that the rate at which it clusters varies markedly with the rate of cooling the milk after it is drawn. On a cold day we could hardly mount the sample before it had clustered although the microscope was set up within 20 feet of the cow being milked. Following the same procedure on a warm day clustering did not take place for two to five minutes after the milk was drawn.

The statement is frequently found in the literature that homogenized milk does not cream and the reason given for this failure to cream is that the fat globules are so broken up that they rise extremely slowly. A series of observations have shown that the fat globules in homogenized milk are not clumped so the absence of clumps accounts for the failure of such milk to cream. It is also common knowledge that the fat globules in homogenized cream are clumped. If milk of average fat content is prepared by adding skim milk to homogenized cream, and this milk is allowed to cream a very deep cream layer is formed and the fat globules are present in the form of very large irregular clusters. This milk will stand vigorous shaking and pasteurization without destroying its creaming ability. This procedure has been used commercially to deepen the cream layer on market milk. Doan (11) in a recent paper has published results which are in agreement with our own experimental observations. He describes a procedure for recognizing the presence of homogenized cream in market milk.

#### PACKING OF FAT IN GRAVITY CREAM

A second phase of gravity creaming is the way in which the fat globules are packed in the cream layer. Assuming that the same amount of fat has risen into the cream, the extent of packing of the fat globules in the cream layer regulates its depth as well as its percentage of fat.

Van Dam and Sirks (33) concluded that clumping and creaming were controlled by the properties of the milk plasma and were independent of the fat. Palmer and Anderson (26) came to a

similar conclusion in regard to creaming and in addition believed that the viscosity of raw milk could be used as an indication of its creaming ability. They disregarded, however, the effect of the presence or absence of clumps on the viscosity, the effect of which was shown to be of considerable importance by Babcock and his coworkers. Palmer, Hening and Anderson (27) made the important discovery that casein hindered creaming and that the whey colloids promoted both cream rising and satisfactory cream volumes. Hekma (18) found that milk prepared from washed cream and centrifugally separated skim milk creamed better than when gravity separated skim milk was used in its preparation. The relative creaming relation was the same if the washed cream was previously heated at 90°C. for five minutes although the cream layers were not so deep.

The addition of various substances to milk has been shown to increase the creaming and the depth of the cream layer of milk. Clausnizer (9) and Babcock (2) found that adding alkali to milk increased its viscosity and the depth of the cream layer and also caused more rapid and complete creaming. Van Dam and Sirks (33) found that the creaming of a poorly creaming milk was favorably influenced by the addition of the proper amount of alkali.

Hammer (15) added viscogen and found that tremendously deep cream layers were formed, often they extended nearly to the bottom of the creaming cylinder. The pronounced clumping thus obtained was associated with deep cream layers of low fat content.

Rahn (28) showed that the addition of gelatin and gum arabic to milk increased the depth of the cream layer, decreased the percentage of fat in the cream, and caused more exhaustive creaming. Van Dam and Sirks (33) obtained similar results with gum tragacanth, saleb and linseed extract. Babcock (2) added blood serum to milk and found that it caused a clumping of the fat globules. Similar results were also obtained with an artificial emulsion of cotton seed oil. Very important contributions to the creaming of milk have resulted from the investigations carried out at the Hoorn Experiment Station in Holland.



These investigations were directed primarily at an explanation for the fact that the milk from some cows creams well while the milk from other cows does not. Since the milk is elaborated by the mammary gland from materials brought to it by the blood it was conceivable at least that the blood serum from cows whose milk creamed well when added to poor creaming milk would influence the creaming more favorably than the blood serum from cows whose milk creamed poorly. Van Dam, Hekma and Sirks (34) failed to establish such a relation and Brouwer (6) was unable to show any difference in the beneficial effect on creaming of adding the blood serum from steers as compared with cows. He was able to show however, that the beneficial effect was due to the euglobulin fraction of the serum. He demonstrated this in two ways first, by showing that the euglobulin was the only constituent of the blood serum which, when added to milk, produced the marked increase in creaming ability and second, by showing that the blood serum of new born calves exerted no appreciable beneficial effect on creaming and contained no euglobulin. This was taken to indicate also that it was the euglobulin itself which was exerting the effect and not materials carried with it by adsorption. Sirks (31) showed that the electrical charge on the fat globule was very low and its magnitude was not correlated with the clumping.

It is well known that when creaming takes place in a normal manner at low temperature, the fat rises in a few hours or less to give a cream containing about 20 per cent fat, and that after standing the cream layer decreases slowly and the fat content of the cream increases. Any explanation of creaming must account for the marked pause in the packing of the fat globules at a fat concentration of about 20 per cent. The most reasonable explanation is that the clumps pack in the cream with spaces between them containing skim milk. If this explanation is not accepted then some other mechanism must be postulated for keeping the fat globules apart. Evidence against some of these alternate explanations will be presented.

Hunziker (21) wrote, "it is necessary to handle the cream in such a manner as to cause the fat globules to rise to the surface

exhaustively and particularly also to insure the carrying up into, and holding in the cream line the maximum amount of non-fatty constituents."

If the fat globules are surrounded by adsorbed layers of sufficient thickness to limit the fat content of the cream to 20 per cent fat, the thickness of such a layer is easily calculated with approximate accuracy. In making such calculations it is necessary to assume that the fat globules of milk are all of the same size, an assumption which is of course not true but the error introduced is probably very small as will be shown later.

Uniform solid spheres may be packed in space according to three regular systems in which the spheres are in contact with their neighbors. Each system is designated according to the figure in which the sphere may be assumed to be inscribed, the sphere being in contact with a neighboring sphere at the central point of each surface. This figure may be a cube, a hexagonal cylinder, or a dodecagon. In the system of cubical packing the sphere occupies 52.36 per cent of the total volume, in the hexagonal cylindrical packing the sphere occupies 60.4 per cent of the total volume, and in the system of dodecagonal packing the sphere occupies 74.04 per cent of the total volume. In this last named system of packing the spheres occupy the maximum of the total volume which it is possible for uniform spheres to occupy.

If it is assumed that the fat globules are not clumped and are of uniform size, they should pack themselves in cream until each sphere touches its neighbors. If we assume that this packing is according to one of the regular systems described, then we should expect the fat content of the cream to be as high as these calculations indicate unless the fat globules are held apart by some means.

It might be supposed that the adsorbed layer of protein material and water on the surface of the fat globules is of such thickness as to hold the fat globules apart a sufficient distance to account for the low fat content of gravity cream. If this supposition is correct it is possible to calculate the thickness the adsorbed layer would have to be to account for the normal fat content of gravity cream, by means of the following equations, the one to use de-

pending on which condition of packing the fat globules in the cream are assumed to take.

$$\text{Cubical packing} \quad x = r \left( \sqrt[3]{\frac{52.36}{V}} - 1 \right) \quad (6)$$

$$\text{Hexagonal cylindrical packing} \quad x = r \left( \sqrt[3]{\frac{60.4}{V}} - 1 \right) \quad (7)$$

$$\text{Dodecagonal packing} \quad x = r \left( \sqrt[3]{\frac{74.04}{V}} - 1 \right) \quad (8)$$

Where  $x$  is the thickness of the adsorbed layer,  $r$  is the radius of the fat globule and  $V$  is the percentage of the total volume occupied by the fat.

Since cream of 20 per cent fat content is representative of gravity cream the calculations were made on the basis that the fat occupied about 21.8 per cent of the volume of the cream. The thickness of adsorbed layers necessary to account for cream of this fat content and the nearest possible approach of the fat globules to each other is given in table 7, in which an average diameter of  $4\mu$  was assumed for the fat globules.

Table 7 indicates that if adsorbed layers are to explain the low fat content in cream they must be abnormally thick. A survey of the literature fails to show that anyone has considered the adsorbed layers to be this thick. If the adsorbed layers are as thick as indicated by table 7 they could be recognized under the microscope for it would be impossible for two fat globules to approach each other nearer than the distances indicated in the table. Microscopic observations indicate that the fat globules are able to approach each other within the resolving power of the microscope, that is, the distance between them is not over  $0.2\mu$ . Further evidence that the adsorbed layers cannot be as thick as the calculations in table 7 indicate, was obtained by packing the fat globules as closely as possible by means of a centrifuge.

Since uniform spheres can be made to occupy 74.04 per cent of a total volume, it should be possible to pack fat globules of uniform size so that they occupy this fraction of the total volume

provided they have no adsorbed layers to keep them apart. If the fat cannot be made to occupy this percentage of the total volume the presence of adsorbed layers would be indicated and the data for the maximum fat content actually obtained, as compared with the limiting value of 74.04, would permit the calculation of the thickness of adsorbed layers necessary to account for this difference.

The highest fat content which could be obtained in cream without destroying or distorting the fat globules was determined. The packing was accomplished by means of an electrically driven laboratory centrifuge. A number of preliminary experiments were performed in developing a method that would give consist-

TABLE 7

*The systems for the packing of uniform spheres, the percentage of the total volume occupied by the spheres in such systems and the distance between fat globules if packed in 20 per cent cream according to each system*

An average diameter of  $4\mu$  was assumed for the fat globules

POLYGON CIRCUMSCRIBED ABOUT THE SPHERE	PERCENTAGE OF TOTAL VOL- UME OCCUPIED BY THE SPHERE	THICKNESS OF ADSORBED LAYER	DISTANCE BETWEEN FAT GLOBULES
	per cent	$\mu$	$\mu$
Cube.....	52.35	0.69	1.38
Hexagonal cylinder.....	60.4	0.80	1.60
Dodecagon.....	74.04	1.01	2.02

ent results, as each of several factors exerted an influence. Some of these factors were the use of clumped or unclumped cream, percentage of fat present, methods of heating, cooling, aging and concentrating the cream and other details of operation. It was finally found best to use cream of about 20 per cent fat content. In order to make sure that the fat globules would be completely solid and not deformable, it was necessary to age the cream for a time at a temperature near  $0^{\circ}\text{C}$ . It was also necessary to carry out the centrifuging at near  $0^{\circ}\text{C}$ . therefore the centrifuging was carried out in a room held at approximately that temperature.

One of the experiments carried out with raw cream containing clumped fat globules will be described in detail here. Experi-

ments of a similar nature with heated cream and with unclumped raw cream are described in the next section of this paper. Two samples of milk from Holstein cows and two from Guernsey cows were cooled in ice water immediately after milking. They were held at a temperature below  $3^{\circ}\text{C}$ . for twenty hours. Most of the cream was then removed with a small dipper. Each sample was standardized to 20 per cent fat with its respective skim milk. The cream was held at a temperature below  $3^{\circ}\text{C}$ . The cream was centrifuged for thirty minutes. During this time the heat of the electric motor warmed the cream up to about  $5^{\circ}\text{C}$ . The cream was centrifuged in glass tubes which held about 45 cc. The cream layer which was tested for fat was subjected to a force of about 810 times the force of gravity. In testing the cream 2.5 grams were taken from the surface and weighed into a Gerber cream test bottle and the test was then completed.

The fat tests were all made very carefully by the Gerber method. Immediate previous experiments (see Dahlberg, Holm, and Troy (10)) showed that the Gerber test as made agreed within a fraction of a per cent with the Roesse-Gottlieb method. If anything the probability is that the Gerber test was a fraction of a percent lower than the Roesse-Gottlieb. The data obtained are given in table 8.

Referring to equations (6) (7) and (8) we see that the fat content of the cream is greater than can be accounted for by the various systems of packing except that in which the spheres occupy 74.04 per cent of the total volume. Substituting 64.5 for the  $V$  in equation (8) and assuming the fat globules are  $4\mu$  in diameter we obtain a value for the thickness of the film of  $94\text{ m}\mu = 0.094\mu$ . This value does not even approach the true thickness of the film for the cream was very well clumped before centrifuging and since the clumps are broken up with greater difficulty at low temperatures some of these clumps still exerted some effect in preventing maximum packing. That the unclumped fat globules can be packed until the fat occupies 72 per cent of the total volume, which indicates an adsorbed layer of  $19\text{ m}\mu$  in thickness will be shown later in this paper.

These experiments indicate that the mechanism which causes

the low fat content of gravity cream, cannot be thick adsorbed layers which hold all globules a definite distance from each other.

The low fat content of the gravity cream cannot be explained by Brownian movement keeping the globules apart. This might be a factor if the fat globules were much smaller but the average fat globule when not near other globules hardly travels further than 1 to  $2\mu$  in its sudden movements. These movements cease entirely, due to each globule coming in contact with its neighbors, as the unclumped globules pack in a cream layer, except an occasional small globule which is surrounded by much larger ones so that it has a little free space. If Brownian movement is the factor which keeps the fat globules apart the cream

TABLE 8

*Maximum percentage of the total volume occupied by fat obtained by packing raw cream (well clumped fat globules) in a centrifuge at a temperature of 5°C.*

SAMPLE NUMBER	BREED	PERCENTAGE OF TOTAL VOLUME OCCUPIED BY THE FAT AFTER AGING THE CREAM	
		1 hour	24 hours
1	Holstein	64	64
2	Holstein	65	65
3	Guernsey	64	64
4	Guernsey	65	65

layer would be deeper the higher the temperature, because Brownian movement increases with a rise in temperature and with a fall in viscosity. It is common knowledge that the depth of the cream layer decreases as the creaming temperature is raised.

Palmer and Anderson (26) found a remarkable relationship between the fat content of the cream, the depth of the cream layer and the viscosity of raw milk. They concluded that the viscosity of the skim milk was an important factor in regulating the depth of the cream layer. Van Dam and Sirks (33) found that the addition of small amounts of lactic acid to milk caused no appreciable change in the viscosity but markedly decreased the creaming. That the clumping of the fat globules and not the

viscosity of the skim milk per se controls the fat content of the cream, can be shown by carefully diluting a sample of well clumped milk with an equal volume of cold water and comparing the volume of cream with that on the undiluted sample. With samples of milk in which the fat was clumped in definite tenacious clumps, the volume of cream obtained by the two methods was found to be essentially the same and the fat in the cream was about equal in both cases. If the cream volume is directly related to the viscosity of the plasma phase the cream volume should have been greatly reduced since the viscosity of the plasma was greatly reduced. The diluted milk was creamed in a cylinder twice the height of the cylinder in which the undiluted raw milk was creamed so that they both contained the same actual amount of whole milk and the cream layers formed were of the same depth. The dilution with water caused a slightly greater loss of fat in the skim milk, although the percentage of fat in the skim milk of the diluted samples was less.

Since the assumption, that an adsorbed layer keeps the fat globules apart in gravity creaming, requires the postulation of absurdly thick layers which could easily be recognized by other methods, and since Brownian movement is not strong enough to keep the fat globules apart and operates in the opposite direction from normal creaming behavior, we are led to adopt the explanation that normal gravity cream is composed of clusters with spaces between the clusters containing plasma with little fat. This explanation was proposed by Rahn (28) and van Dam and Sirks (33).

The calculations based on Stokes' law and the experimental verification of them indicate that the cream rises not as individual fat globules but as clusters. The calculations, made from the curve indicated by the experimental data, for the rate of rise of clusters in which the fat was densely packed indicate that slightly less than 50 per cent of the volume of such clusters was occupied by fat.

In gravity creaming these clusters, each containing about 48 per cent of fat by volume, would probably form cream by grouping themselves somewhat similar to the packing of the spheres,

and thus by superimposing a double system of spherical packing of globules into clusters, and these clusters into cream, the volume of fat in the cream would probably be indicated approximately by applying the 48 per cent packing figures twice, giving about 23 per cent of the volume of the cream as fat, or about 21 per cent by weight. This leads to a value rather close to the fat content of normal gravity cream.

Actual experimental evidence has been obtained with microscopic creaming slides, that this is the method of packing of the fat in the cream. The fat was observed to rise in the form of clusters, and these clusters held their form in the cream and enclosed between them considerable volumes of plasma which contained a few unclumped fat globules. Thus the fat content of the gravity cream was found to be regulated by the clusters. If the clusters were large and irregular, they tended to produce cream of low fat content but large volume for a given amount of fat. If milk heated above 70°C. for thirty minutes was allowed to cream in the microscopic chamber, it was observed that the fat globules rose as individuals, and that they packed themselves closely together in the cream. No large spaces free from fat were observed. Such milk required days to cream in a cylinder and the cream was found to contain a high percentage of fat.

Plate 2 A is a microphotograph through a thin cream layer in the microscopic creaming chamber. This photograph shows clearly how the clumps inclose between them volumes of plasma. Plate 2 B shows the bottom edge of a relatively much thicker cross section of a cream layer. In this photograph the lighting was more intense so that the individual globules do not show but here also the volumes containing little fat can be readily seen as light areas. Also the bottom edge of the cream layer is very irregular. It was not thought worth while to take a photograph of the cream layer obtained from non-clumped fat globules. If the attempt were made to take such a photograph through the cream layer of the unclumped fat to correspond to plate 2 A the picture would be absolutely all black. If a photograph of the bottom edge were taken the result would have been a picture with the upper half absolutely black and the lower half completely



white. In this case the bottom edge of the cream would be a straight line dividing the dark and light portions.

A number of samples of colostrum milk were examined for their creaming ability. The main data obtained will be presented in another paper, but one of the samples showed such striking behavior that it seems of interest to mention it here. This sample of milk contained 0.5 per cent fat, 18.2 per cent total solids, and 13.7 per cent protein. It formed 10.5 per cent of its volume as cream; the cream contained 4.3 per cent fat; and the skim milk contained 0.04 per cent fat, determined by Mojonnier method. The cream line formed on this milk was very definite and clear cut, and could be seen with greater ease than the cream layer on normal milk. This cream would form again in ten to fifteen minutes after gently mixing the sample. The clumps were so large that they could be seen through the walls of an ordinary milk bottle. The viscosity of the whole milk at 25°C. was 6.49 centipoise, and of the skim milk 6.16 centipoise. We have found other samples of colostrum milk which showed marked creaming ability, but we have found no others so far which equalled the sample described here.

Hammer (15) reported that the addition of viscogen to milk in some cases caused the cream to occupy nearly the entire volume of the creaming cylinder. The clumps could be observed with the unaided eye.

Another case of very marked irregular clumps forming a deep cream layer of low fat content is the creaming of milk prepared from skim milk and well clumped homogenized cream. Such milk will frequently give about three-fourths of its volume as cream and the cream may contain as low as 5 to 6 per cent of fat.

Palmer, Hening, and Anderson (27) made the very important discovery that if high fat content cream was added to whey to give about the fat content found in normal milk such whey milk showed good creaming properties. We have confirmed their experiment and have shown in addition that the whey produces clumping of the fat globules. In one of our experiments 50 per cent raw cream was added to rennet whey (which had been previously pasteurized at 60°C. to inactivate the rennet), to make

a 4.5 per cent fat content and the mixture pasteurized. At the end of twenty-four hours a cream volume of 16.3 per cent was obtained. The skim whey contained 0.35 per cent of fat. The fat globules were clumped as shown in plate 3 A. When instead of raw cream, cream which had been heated to 78°C. for fifteen minutes was used in making the whey milk the cream layer occupied 10 per cent of the volume and the skim whey contained 1.9 per cent of fat. In this case the fat globules were also clumped to a considerable extent although the clumping was not so complete nor were the clumps so large as when raw cream was used. The clumps are shown in plate 3 B. Thus the creaming of whey milk is in accord with the clumping of the fat globules.

#### PASTEURIZED MILK

It is well known that if milk is heated for too long a time or at too high a temperature it creams poorly. Babcock and Russell (3), Farrington and Russell (13), Kersten (22), Hammer and Hauser (16), Kilbourne (23), Hammer (15), Burri (7), Hunziker (21), Harding (17) and Whittaker, Archibald, Shere and Clement (38) have shown that the pasteurization must be done very carefully or the milk will be heated to the extent that the creaming is interfered with. Babcock and Russell (3), Rahn (28) and van Dam and Sirks (33) have shown that severely heated milk contains no clumps of fat globules when the milk is cooled. Babcock and Russell stated that the limit beyond which milk could not be pasteurized without destroying the clumps was between 59° and 65°C.

During the pasteurization of milk by the holding process at normal pasteurization temperature the clumps of fat globules which were originally present in the raw milk are broken up. Milk was taken from a commercial pasteurizer at the end of pasteurization but before cooling. The sample was taken to a room whose temperature was 35°C. and mounted in the microscopic creaming cell. The milk contained no clumps of more than 6 to 8 globules nor did larger clumps form during about eight hours of periodic observation. The great majority of the fat globules were not clumped at all and the small clumps which

were observed are of little importance in creaming. The creaming occurred extremely slowly as would be expected in unclumped milk. Bottles of milk taken from the pasteurizer after pasteurization and before cooling were set to cream at 45°, 35° and 0°C. Only a trace of cream was observed on the samples creamed at 45° and 35° but a normal cream layer was obtained on the sample creamed at 0°C. If, however, the pasteurization temperature is too high the fat globules fail to clump again when the milk is cooled in the customary manner, and the milk fails to give cream in a reasonable time. Thus the detrimental effect of pasteurization on creaming is due to heating the milk to the extent that the fat globules do not clump again on cooling. Some times pasteurized milk is cooled with so much agitation that the clumps which are formed during the cooling are broken up and consequently the milk creams poorly but this is not the fault of the pasteurization since the creaming of raw milk would be injured by similar agitation.

Our experiments have shown that if the pasteurization of the milk and subsequent treatment is such that it gives normal gravity cream the fat globules clump again before creaming occurs. The microphotographs in plates 1, 2 and 3 were all obtained with milk which had been pasteurized.

Roberts (30) Hammer and Hauser (16) Burri (7) van Dam, Hekma and Sirks (34) and Whittaker, Archibald, Shere and Clement (38) have observed that low temperature pasteurization often improves the creaming of milk. This behavior can be explained by our observations that if the milk is agitated, especially at near room temperature or below, the clumps of fat globules are broken down and they do not readily form again even if the milk is then cooled to a low temperature. If, however, after the clumps in raw milk have been broken down at near room temperature, the milk is then carefully pasteurized and cooled rapidly to a low temperature with not too much agitation, the large clumps form again and an improvement in the cream layer is observed over that of the raw milk which has been agitated at room temperature. If the raw milk as soon as it is drawn is immediately cooled rapidly to a low temperature with not too

much agitation it will give a cream layer which will not be materially improved by low temperature pasteurization. The fact that the creaming of the raw milk is improved by pasteurization probably in most cases indicates that the raw milk has been treated in such a way during or after cooling as to break up the clumps of fat globules or to prevent their forming. The cream obtained on such raw milk does not represent the true maximum creaming ability of which it would have been capable as raw milk if properly treated.

It has been suggested by Kirchner (24) Eichloff (12) Hunziker (21) and others that the reason heated milk does not cream is because the albumin is coagulated on the surface of the fat globules and thus weights them so that they do not rise, or rise only slowly. This explanation is not adequate to account for the failure of heated milk to cream as can be shown in various ways. In the first place there is not enough albumin in milk to sufficiently weight the fat globules so as to prevent their rising. If the density of the fat at room temperature is taken as 0.921, of skim milk 1.032, and of albumin 1.275, (Chick and Martin (8) found that the density of dry serum albumin was 1.275) it can be calculated that the layer necessary to weight a fat globule so that it will not rise is  $0.134 r$ , where  $r$  is the radius of the fat globule in  $\mu$ . The layer on a fat globule  $4\mu$  in diameter would have to be  $0.268\mu$  thick and the closest such globules could come to each other would be  $0.536\mu$ . This would increase the effective volume of the fat globules by about 45 per cent. A milk containing 4 per cent of fat would have to contain 2.52 per cent of albumin to produce the weighting. The amount of heat coagulable protein in milk is usually about 0.2 to 0.3 per cent. These calculations of the thickness of the coagulated adsorbed layer are based on the assumption that it is not hydrated. Since such a layer would undoubtedly be hydrated, its actual thickness, if it formed, would be much greater.

The postulate (Hunziker (21)) that in heated milk a net work structure is formed which prevents the fat globules from rising is not tenable because the viscosity of skim milk which has previously been heated to  $70^{\circ}\text{C}$ . for thirty minutes is less than

the viscosity of the unheated milk as shown by Whitaker, Sherman and Sharp (37). This would not be true if such heating produced a structure which would retard the rising of fat globules for such a structure would also increase the viscosity. This heat treatment of milk markedly reduces the creaming ability of whole milk. The viscosity of whole milk heated under these conditions is also less than the viscosity of the unheated milk.

If coagulation of the protein on the surface of the fat globules produced a layer of sufficient thickness to prevent the rising of cream, the layer could be detected by the packing of solid fat globules by means of the centrifuge. Packing experiments using heated cream were made in a manner similar to those shown in table 8 for raw milk.

TABLE 9

*Maximum packing of fat globules in cream which had been heated, and cooled for various periods of time before packing*

SAMPLE NUMBER	FAT CONTENT OF CREAM BY PERCENTAGE VOLUME AFTER COOLING FOR		
	2 hours	5 hours	22 hours
1	73.9	71.9	71.9
2	73.9	71.9	71.9
3	75.0*	71.9	71.9

\* Centrifuged one hour after cooling.

The cream used was obtained by centrifugal separation from the pasteurized milk of the college herd. The herd contained Holstein, Guernsey, Jersey and Ayrshire cows. The cream was standardized to 20 per cent fat. About 200 cc. was then placed in a 250 cc. Erlenmeyer flask and the flask was placed in a water bath at 72.2°C. (162°F.) for a period of thirty minutes. The cream was stirred constantly by means of a bent glass rod attached to an electric motor. The stirrer revolved fast enough to keep all of the cream in slow motion but not fast enough to whip any air into it. When the heating was completed the flask was placed in ice water and cooled to a temperature below 3°C. Milk of normal fat content made from this heated cream and skim milk

subjected to the same heat treatment fails to cream in a reasonable time. At the end of one hour of cooling the cream was filtered through a layer of cotton about  $\frac{3}{4}$  inch in thickness in order to remove any congealed particles resulting from the small amount of fat oiling off during the heating process. Part of the cream was centrifuged two hours after cooling; a second part five hours later, and a third part twenty-two hours later. The centrifuging was for thirty minutes and was done in a cold room as previously described. The data are given in table 9. Each experiment was performed on a different day with a different sample of milk. The higher fat content obtained at the end of one and two hours was probably due to the fat in the globules being still partly liquid so that some of the globules were deformed in the packing process. After five hours, however, a constant lower value was reached. If we assume that the fat globules are all uniform spheres the maximum limit is 74.04 per cent for the volume of the fat, actually 72 per cent was obtained, using 0.961 as the density of the fat at 5°C., that is 2 per cent less than the limit. This experiment indicates that the unclumped fat globules were packed according to the system of maximum packing of spheres. If the thickness of an adsorbed layer necessary to account for this difference of 2 per cent is calculated by Equation (8) we find that for globules of  $4\mu$  diameter the layer should be  $19\text{ m}\mu$  ( $0.019\mu$ ) thick. That is about one-tenth of the thickness it would have to be, if it were composed of albumin with a density of 1.275, in order to prevent the creaming of heated milk.

Rahn (28) has concluded from measuring the actual rates of rise of individual fat globules from heated and unheated milk that they rise at approximately the same rate.

The difference in the packing in tables 7 and 9 is not due to a difference in the thickness of an adsorbed layer but is due to some of the clumps in table 7 still offering resistance to packing. This was shown by using a different method for breaking up the clumps, namely, by forcing raw cream at a temperature below 3°C. through a capillary several times without introducing air into it. The results obtained are indicated in table 10.

We believe that it is probable that the layer on the fat globules

is less than  $19\text{ m}\mu$  in both raw and heated milk and also that it is not composed of materials of as high a density as 1.275 otherwise a layer of even the thickness of  $19\text{ m}\mu$  would cause the fat globules to rise appreciably more slowly than the values obtained in table 3, and it is probable that we would have detected such an effect. Since the experimental rates of rise agree so well with the calculated rate when no correction is made for the weighting effect of the adsorbed layer, it is probable that either the layer is not of

TABLE 10

*Maximum packing of solid fat globules by means of a centrifuge in clumped and non-clumped raw cream*

The cream was held at near  $0^{\circ}\text{C}.$  for twenty-four hours before being centrifuged.

SEMED	TIMES THROUGH THE CAPILLARY	FAT CONTENT OF CREAM BY VOLUME
		<i>per cent</i>
Holstein sample no. 1.....	0	65.6
	0	64.6
	10	70.3
	10	69.8
Holstein sample no. 2.....	0	66.7
	0	66.7
	15	70.8
	15	71.9
Jersey sample no. 3.....	0	67.7
	0	67.7
	15	72.9
	15	71.9

great thickness or it does not have a very high density. The value of  $19\text{ m}\mu$  is possibly too high as based on the fat test.

#### SUMMARY AND CONCLUSIONS

1. The rate of rise in milk plasma of individual fat globules varying in size from  $1.8$  to  $41\mu$  in diameter was determined. The experimental results were found to agree very well with the values calculated by Stokes' equation.

2. The fat globules rise so slowly as independent individuals that it would require many times the normal creaming time of milk for them to reach the cream layer.

3. The rate of rise in milk plasma of fat globule clusters varying in size from 10 to 800 $\mu$  in diameter was determined. The experimental results were found to agree very well with the values calculated by Stokes' equation.

4. The dense spherical clusters probably contain slightly less than 50 per cent of fat by volume.

5. It is shown that clusters rise rapidly enough to account for the normal creaming time.

6. Clusters of sufficient size to account for the normal creaming time of milk were observed in normal raw milk and carefully pasteurized milk.

7. It was found that the clusters arranged themselves in the cream layer with volumes of plasma relatively free from fat between them. The packing of the fat globules into clusters, and then of the clusters in the cream, with volumes free from fat between them, readily explains the low fat content of gravity cream.

The conclusion is drawn, that, for a given percentage of fat, the depth of the cream layer depends primarily on the clustering of the fat globules. Large irregular stable clusters form deep cream layers. Compact approximately spherical clusters, and especially weak clusters, form shallow layers and the fat content of the cream is high.

8. The rigidity of the clusters gradually lessens as the creaming temperature increases permitting closer packing of the clusters at the higher temperatures. This explains why the deeper cream layers are obtained at the lower temperatures. If the creaming temperature is too high the clusters do not form at all or, if formed at a colder temperature, they tend to disintegrate on warming.

9. The stability of the clusters in several instances was found to vary with the sample of milk and its treatment.

10. Mechanical agitation was found to break up the clusters especially at near room temperature.

11. Unclustered fat globules were found to pack themselves



in the cream layer very closely. The fat content of such cream was very high and the cream required days to form.

12. A microscopic creaming cell was described and it was pointed out that in order to compare definitely the creaming of a bulk sample of milk with clustering, it is necessary to carry out the creaming with the microscopic creaming cell under the same conditions as with the bulk sample.

13. It was pointed out that creaming time may be divided into two parts; first, the time required for the clusters to form, and second, the time required for the clusters to rise after they have formed.

14. The thickness of the adsorbed layer on the fat globules of raw milk and milk that had been heated to a temperature which destroyed its clustering and normal creaming power was shown to be the same in as far as this could be determined by the maximum packing of the fat globules by means of a centrifuge.

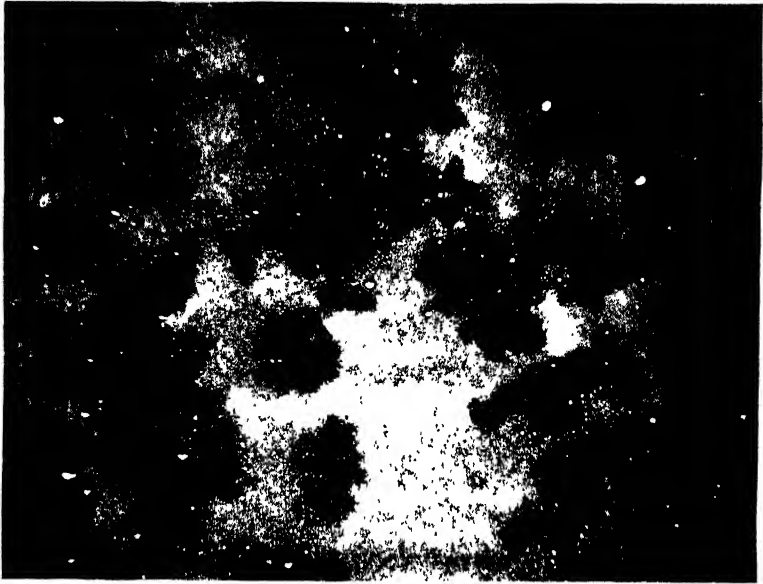
15. The maximum packing of the fat globules indicated that the adsorbed layer was about 19  $m\mu$  in thickness.

#### REFERENCES

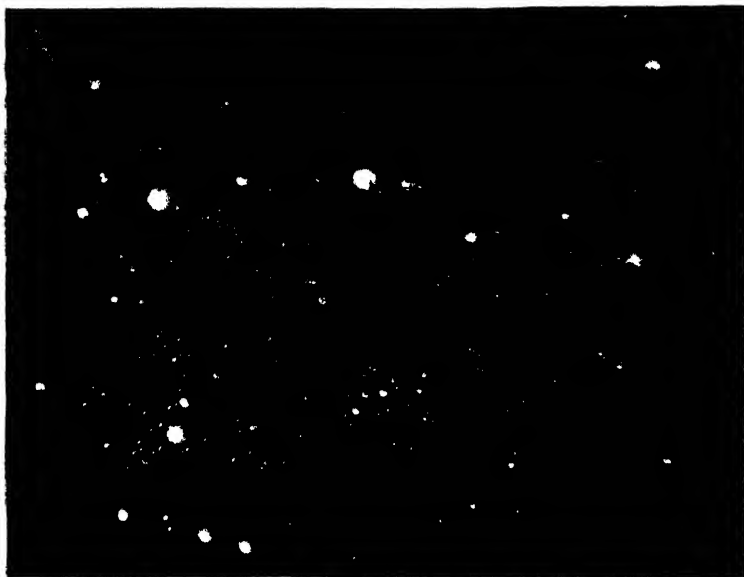
- (1) ARNOLD, L. B. 1879 American Dairying. Rural Home Company, Rochester, N. Y.
- (2) BABCOCK, S. M. 1899 The constitution of milk, and some of the conditions which affect the separation of cream. Wisc. Agr. Expt. Sta. Bul. No. 18.
- (3) BABCOCK, S. M., AND RUSSELL, H. L. 1896 Conditions affecting the consistency of milk. Wisc. Agr. Expt. Sta. 13th Ann. Rpt., 73-80.
- (4) BABCOCK, S. M., AND RUSSELL, H. L. 1896 On the restoration of the consistency of pasteurized milk and cream. Wisc. Agr. Expt. Sta. 13th Ann. Rpt., 81-94.
- (5) BANCROFT, W. D. 1921 Applied Colloid Chemistry. McGraw-Hill Book Company, New York.
- (6) BROUWER, E. 1925 Over het wezen der vetbolletjes-agglutinatie. IV. Over serumglobuline in verband met de melkoprooming. Vers. landb. onderzoek. Rijkslandbouw. No. 30, 261-278.
- (7) BURRI, R. 1916 Über die Beeinflussung des Aufrahmungsvermögens durch eine vorausgegangene Erwärmung der Milch. Milchw. Zentralb., xlv, 33-39.
- (8) CHICK, H., AND MARTIN, C. J. 1913 The density and solution volume of some proteins. Biochem. Jour., vii, 92-96.
- (9) CLAUSNITZER, F. 1879 Ein neues Aufrahmungs-Verfahren. Milch-Zeitg., viii, 510-513.

- (10) DAHLBERG, A. C., HOLM, G. E., AND TROY, H. C. 1926 A comparison of the Babcock, Gerber, and Roese-Gottlieb methods for determining the percentage of fat in milk and cream. N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. No. 122.
- (11) DOAN, F. J. 1927 "Viscolized" milk and its Detection. Jour. Dairy Sci., x, 501-512.
- (12) EICHLOFF, R. 1916 Massnahmen zur Erreichung der höchsten Butterausbente. Milchw. Zentralb., xlv, 379-383.
- (13) FARRINGTON, E. H., AND RUSSELL, H. L. 1899 Pasteurization of milk and cream at 140°F. Wisc. Agr. Expt. Sta. 16th Ann. Rpt., 129-139.
- (14) FLEISCHMANN, W. 1893 Lehrbuch der Milchwirtschaft. Bremen.
- (15) HAMMER, B. W. 1916 Studies on the creaming ability of milk. Iowa Agr. Expt. Sta. Res. Bul. No. 31.
- (16) HAMMER, B. W., AND HAUSER, A. J. 1914 The pasteurization of milk in the final package. Iowa Agr. Expt. Sta. Bul. No. 154.
- (17) HARDING, H. A. 1921 Effect of temperature of pasteurization on the creaming ability of milk. Ill. Agr. Expt. Sta. Bul. 237.
- (18) HEKMA, E. 1925 Over het wezen der vetbolletjes-agglutinatie. V. Vergelijkend onderzoek omtrent den invloed van schep- en centrifugeondermelk op de oprooming van gewasschen melkvetbolletjes. Vers. landb. onderzoek. Rijkslandbouw., No. 30, 279-286.
- (19) HEKMA, E., AND SIRKS, H. A. 1924 Over het wezen der vetbolletjes-agglutinatie. Vers. landb. onderzoek. Rijkslandbouw., No. 29, 94-109.
- (20) HENSEVAL, 1902 Étude sur les laits à ascension rapide et lente. Rev. gén. du lait., i, 366-373.
- (21) HUNZIKER, O. F. 1921 Pasteurization and the cream line. Hoard's Dairyman., lxi, 91.
- (22) KERSTEN, M. 1911 Über das Aufrahmen pasteurisierter Milch. Molkerei-Ztg. (Hildesheim), xxv, 553-555, 567-569.
- (23) KILBOURNE, C. H. 1915 What causes contribute to the loss of the cream line on pasteurized milk in bottles. Creamery and Milk Plant Monthly, iii, no. 8, 11-20.
- (24) KIRCHNER, W. 1898 Handbuch der Milchwirtschaft. 4th Ed. 172. Parey. Berlin.
- (25) MARCAS, L. 1903 Contribution a l'étude des laits a ascension lente. Bul. Agr. (Bruxelles), xix, 1228-1234.
- (26) PALMER, L. S., AND ANDERSON, E. O. 1926 Physicochemical factors influencing cream rising. I. Viscosity. Jour. Dairy Sci., ix, 1-14.
- (27) PALMER, L. S., HENING, J. C., AND ANDERSON, E. O. 1926 Physicochemical factors influencing cream rising. II. Relation of plasma colloids to pasteurization effects. Jour. Dairy Sci., ix, 171-191.
- (28) RAHN, O. 1921 Untersuchungen über die Rahmbildung. Forsch. geb. Milchwirtsch. u. Molkereiwesen., i, 133-154, 165-181, 213-233.
- (29) RICHMOND, H. D. 1920 Dairy Chemistry. Charles Griffin & Company, London.
- (30) ROBERTS, I. P. 1889 Does heating milk affect the quality or quantity of butter. Cornell Agr. Expt. Sta. Bul. No. 5, 13-20.

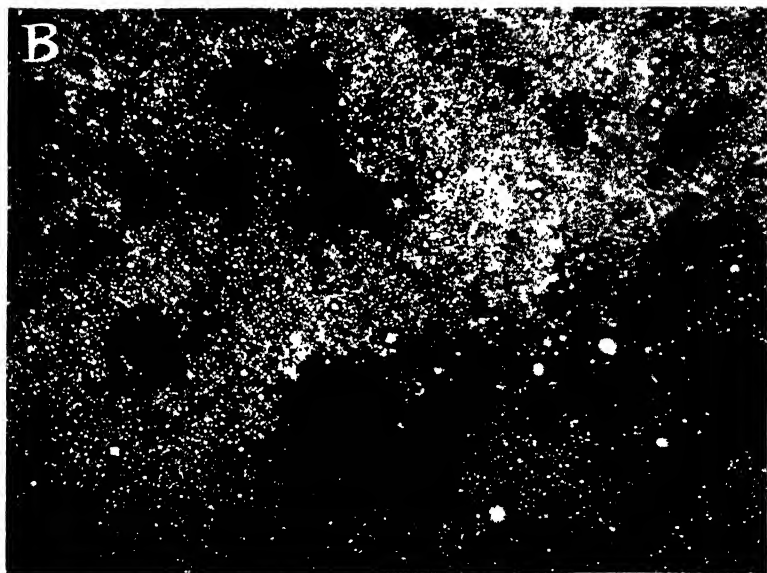
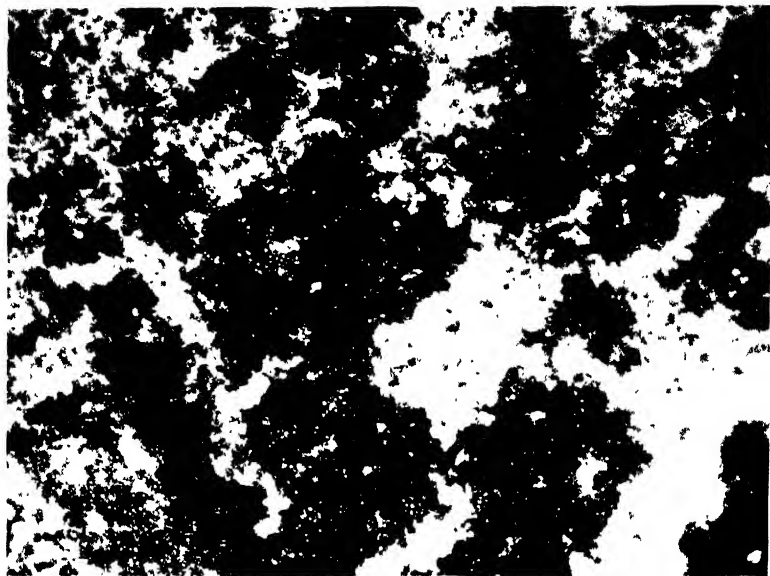
- (31) SIRKS, H. A. 1924 Over het wezen der vetbolletjes-agglutinatie. De agglutinatie der vetbolletjes en hunne electrische lading. Vers. landb. onderzoek. Rijkslandbouw. No. 30, 261-278.
- (32) SVEDBERG, T., AND ESTRUP, K. 1911 Ueber die Bestimmung der Häufigkeitsverteilung der Teilchengrößen in einem dispersen System. Kolloid, Z., ix, 259-261.
- (33) VAN DAM, W., AND SIRKS, H. A. 1922 Onderzoekingen over de oprooming volgens het Froesch systeem. Vers. landb. onderzoek. Rijkslandbouw. No. 26, 106-186.
- (34) VAN DAM, W., HEKMA, E., AND SIRKS, H. A. 1923 Over het wezen der vetbolletjes-agglutinatie. Vers. landb. onderzoek. Rijkslandbouw., No. 28. 100-112.
- (35) VAN DER BURG, B. 1921 De beweging van een vetbolletje in de melk. Het Algemeen Zuivelblad., January 7 (1921), 3-4.
- (36) VAN DER BURG, B. 1927 Etudé du lait considéré comme émulsion. Le processus de la montée de la crème. Le Lait., vii, 452-466.
- (37) WHITAKER, R., SHERMAN, J. M., AND SHARP, P. F. 1927 Effect of temperature on the viscosity of skim milk. Jour. Dairy Sci., x, 361-371.
- (38) WHITTAKER, H. A., ARCHIBALD, R. W., SHERE, L., AND CLEMENT, C. E. 1925 Effect of various factors on the creaming ability of market milk. U. S. D. A. Dept. Bul. 1344.
- (39) WIEGNER, G. 1914 Ueber die Aenderung einiger physikalischer Eigenschaften der Kuhmilch mit der Zerteilung ihrer dispersen Phasen. Kolloid. Z., xv, 105-123.
- (40) WOLL, F. W., BABCOCK, S. M., AND RUSSELL, H. L. 1903 Conditions affecting the consistency of milk; means of restoring consistence of pasteurised cream. Wisc. Agr. Expt. Sta. 20th Ann. Rpt., 183-187.



- A. Clumps of fat globules in pasteurized milk after cooling.  
B. Large nearly spherical dense clump such as was used for measuring the rate of rise.



A. View through a thin layer of cream on cooled pasteurized milk.  
B. Bottom edge of a thin layer of cream on cooled pasteurized milk.



A. Clustering of fat globules when raw cream is added to whey and the mixture is pasteurized and cooled. Magnification 141 times.

B. Clustering of fat globules when heated cream is added to whey and the mixture is pasteurized and cooled. Magnification 141 times.

# THE ACTIVITY OF THE MAMMARY GLAND AS DETERMINED BY ANALYSIS OF MAMMARY BLOOD BEFORE AND AFTER MILKING\*

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There is much difference of opinion among dairymen and students of dairying as to the nature of milk secretion. We would know among other things whether or not milk secretion is a continuous process, proceeding at a uniform rate throughout the period of time between milkings. Does the stimulus of the milker activate secretion or only release the secreted product? Finally is a milk distended udder due mainly to the presence of milk external to the alveolar cells or internal to them? It is our aim in this paper to report the results of work which we believe contribute in a measure to replace theory with fact.

## UDDER CAPACITY, INFLUENCE ON CONTINUITY AND RATE OF SECRETION

The commonly accepted idea that milk is largely secreted at the time milk is being drawn was promulgated by many of the earlier observers. Marshall (1) states that the major part of secretion takes place at milking time because a cow's udder cannot contain the entire production at once. Judkins (2) in his college text elaborates the idea. More recent study indicates that this conception should be modified. Gaines (3), Swett (4), Foa (5), and Roehrig (6) report experiments in measurement, perfusion, and pumping back liquids into the udder which prove very definitely that cows' udders do have capacity even greater than their milk yields. Gaines and Sanmann (7) by quantitative analysis of full udders for lactose add new evidence that milk synthesis is complete at the time of milking.

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Upon these observations the above named workers apparently lean to the idea that milk secretion is more or less uniform in rate throughout any twelve-hour period.

Ragsdale, Turner and Brody (9) have reduced to experimental data the principle long made use of by register of merit dairymen that milking more frequently than semi-daily increases both milk yield and butterfat. They place secretion just after milking at 100 per cent and find a 5 per cent reduction rate per hour thereafter due, they think, to pressure of accumulating secreted product.

Just here it may be well to note that in the advancement of these theories it is not always clear what the writers mean by the term "milk secretion." Bayliss (10) in defining secretion in general lists factors which may be included for brevity under two processes: first, synthesis of the normal product within the secreting cell from materials supplied by the blood; second, liberation through the cell walls of the synthesized product. By this definition milk secretion may be thought of first in terms of synthesis and finally liberation of product. Either milk is liberated from the alveolar cells as synthesized, or it is stored in cell vacuoles until the stimulus of milking sets in motion the reflexes which produce rapid release of the product. The latter conception would seem best to account for the phenomena commonly referred to as "holding up" or "giving down" milk.

#### VARIATION IN COMPOSITION OF MILK AS SECRETED

Jackson and Rothera (11) found milk secreted under different natural conditions to have different electrical conductivity. Davidson (12) found marked changes in milk secreted after the re-injection of sanitarily produced milk into the emptied udder from whence it had just been drawn. Lactose and isotonic salt solutions produced the same results, but distilled water did not. The benefit derived from pumping air into the udder in cases of milk fever may be explained by the production of pressure on the alveoli which stops secretion and thereby cuts off the drain of sugar from the blood. Careful dairymen know that high producing cows should not be milked out completely during the first



flush of lactation. These observations all suggest an uneven rate in milk secretion.

Swett (4) has recently published results on milking excised udders. Yields from such milkings on three cows were approximately 50, 69, and 85 per cent of normal. Turner and Uren at Missouri and Olson, South Dakota, from unpublished data kindly submitted to the author furnished evidence that corroborates in the main Swett's findings. One way to interpret these results is that milk is synthesized and liberated into the lumina, milk ducts, and cisterns to the extent of from 50 to 85 per cent of normal yield at the time of milking. Another interpretation would assume that milk is held in a synthesized state in the alveolar cells, except for a slow leakage of low fat milk known as first milk; and that excised udders are sufficiently responsive to the milking stimulus to liberate their stores to a point when, perhaps through lack of blood circulation, there is insufficient water to perfuse the remaining fraction through the cell walls.

#### BLOOD PRECURSORS OF MILK

In 1906 Kaufman and Magne (13) found sufficient difference in the glucose content of mammary blood and jugular blood to account for the lactose found in milk. On this suggestion Meigs, Blatherwick and Cary (8) found a similar difference in blood phosphatides and inorganic phosphorus to account for milk fat. Cary (14) followed these discoveries with a determination of amino acids in mammary and jugular blood. His results accounted for milk proteins. Hence the precursors of the three principal organic constituents of milk are accounted for.

Since it has been possible for the above named workers to obtain indicative results on milk secretion by blood analysis it occurred to us that analyses of mammary blood before and after milking might throw light on the problem of rate of secretion.

Accordingly we planned to take samples of blood from the mammary veins of lactating cows just before milking. A check sample of jugular blood was also taken immediately after the mammary sample. After milking, another mammary sample of

blood was taken from the same vein as before milking. We used Meigs method of taking sample; namely, by means of inserting a large hypodermic needle into the vein and removing approxi-

TABLE 1

*Inorganic phosphorus of mammary and systemic blood in lactating cows (averages)*  
 Milligram per 100 cc. plasma

NAME OF COW	BEFORE MILKING	AFTER MILKING	SYSTEMIC	SEMI-DAILY MILK YIELD
				<i>pounds</i>
Pauline.....	5.82	5.57	5.29	5-8
Lady.....	5.61	6.06	6.25	5-8
Aloise.....	4.95	4.37	4.96	8-2
Liza.....	5.76	5.39	5.45	7-9
Jones:				
Blood.....	5.52	5.25	5.12	} 22-26
Plasma.....	5.56	5.03	5.11	
Susan:				
Blood.....	5.27	5.04	5.02	} 11-13
Plasma.....	5.42	4.84	5.35	

TABLE 2

*Organic phosphorus of mammary and systemic blood in lactating cows (averages)*  
 Milligram per 100 cc. plasma

NAME OF COW	BEFORE MILKING	AFTER MILKING	SYSTEMIC	SEMI-DAILY MILK YIELD
				<i>pounds</i>
Pauline.....	6.74	6.61	6.62	5-8
Lady.....	7.72	7.49	7.32	5-8
Aloise.....	7.72	7.17	7.43	8-2
Liza:				
Blood.....	7.53	7.30	7.34	} 7-9
Plasma.....	5.56	4.76	5.66	
Jones:				
Blood.....	7.20	6.49	7.06	} 22-26
Plasma.....	5.84	5.26	5.93	
Susan:				
Blood.....	8.57	8.08	8.33	} 11-13
Plasma.....	7.63	7.40	7.32	

mately 100 cc. of blood which was kept from clotting by means of 1 cc. saturated solution of sodium citrate. This blood was used to determine inorganic and organic phosphorus.

Six cows were used in this experiment. Two were Holstein, one Ayrshire, and three Guernsey. They represented low, medium and high production. From ten to twenty blood samples were taken from each cow and analyzed for the two forms of phosphorus. Each sample analysed was done in four parts for accuracy. The Briggs Modification (15) of the Bell Doisy

TABLE 3  
*Daily variations in blood phosphorus*  
Milligram per 100 cc. whole blood

DATE (1927)	INORGANIC PHOSPHORUS			LIPOID PHOSPHORUS		
	Before milking	After milking	Systemic	Before milking	After milking	Systemic
May 31.....	5.18	4.98	4.73	7.54	6.39	7.22
June 1.....	4.46	3.59	3.28	9.69	9.27	8.70
June 2.....	3.41	3.34	2.99	10.03	9.43	9.64
June 3.....	4.50	4.18	4.27	10.33	9.61	10.04
June 4.....	4.48	4.16	4.32	9.85	8.89	9.36

TABLE 4  
*Periodic variation in blood phosphorus after milking*  
Milligram per 100 cc. whole blood

TIME	INORGANIC PHOSPHORUS			LIPOID PHOSPHORUS		
	Before milking	After milking	Systemic	Before milking	After milking	Systemic
4 p.m.....	4.32	3.50	3.50	10.48	10.11	10.40
6 p.m.....		3.66			10.70	
8 p.m.....		4.04			10.54	
10 p.m.....		4.79			10.24	
12 m.....		4.46			10.54	10.65

method was used for inorganic phosphorus determination, and Bloor's (16) method for the determination of lipoid phosphorus. The results show only slight differences, as might be expected, but the preponderance of difference is in favor of the idea that mammary blood before milking is higher than systemic blood in both inorganic and lipoid phosphorus. In after milking mammary blood both forms of phosphorus, in approximately the

same degree, are lower than in systemic blood. Tables 1 and 2 give the total average of each cow.

These results were secured from blood taken at variable intervals of time. Although the ratio between before milking and after milking mammary blood and systemic blood was fairly constant, the quantity of both inorganic and lipid phosphorus from time to time was variable. To determine if this variability was normal or due to some other cause a single cow (Susan) was made a subject for five consecutive days. The results (table 3) show, so far as one trial can be relied upon, that the phosphorus content of blood is subject to daily variations. It would seem from these figures and from previous analyses that lipid phosphorus tends to be high when inorganic phosphorus is low and vice versa.

A similar morning and evening test also shows a small range of variation as shown by the following analysis:

	INORGANIC PHOSPHORUS			LIPID PHOSPHORUS		
	Before	After	Systemic	Before	After	Systemic
a.m.....	4.73	4.62	3.47	9.44	8.64	9.63
p.m.....	4.89	4.55	4.09	10.60	9.84	9.84

Meigs and co-workers found a difference in blood phosphorus content when the blood was taken during the milking act as compared to samples taken when cows were undisturbed. In order to compare the effect of massage on blood phosphorus content as compared with conditions before and after milking, we took samples as usual but just after taking the before milking and systemic samples the udder was thoroughly massaged and another sample taken before milking was begun. Results are given in order of samples taken as follows:

	INORGANIC PHOSPHORUS	LIPID PHOSPHORUS
Before milking.....	5.58	10.58
Systemic.....	4.63	10.80
Massage.....	4.76	9.87
After milking.....	4.32	10.62

Massaging would seem to have some influence in stimulating some form of udder activity which calls for the use or retention of both forms of phosphorus.

If milk secretion proceeds at a diminishing rate as indicated by the production curves of Ragsdale, Turner and Brody it might be possible to trace this diminishing rate through blood analysis. For this test a high producing cow was chosen (Jones). After taking the before, systemic and after milking samples as usual another sample was taken from the mammary vein every two hours thereafter until eight hours had elapsed. The results are presented in table 4.

#### DISCUSSION

The phosphatides (lecithin for example) approximate in molecular composition one part by weight of phosphorus to eighteen parts of fat. In the synthesis of milk fats from phosphatides it is generally accepted that the phosphorus radical is replaced by a fatty acid radical. In the synthesis of 4 per cent milk four parts of fat per hundred are produced, one-eighteenth of which in the lecithin form or 0.224 part was phosphorus. One hundred parts of 4 per cent milk contain in composition 0.086 part of phosphorus (17). If we divide the amount of phosphorus released from lecithin in the production of 100 parts of 4 per cent milk by the amount of phosphorus contained in that milk in its normal composition we have the figure 2.6. Therefore approximately one and one-half times as much phosphorus is released in fat synthesis as is utilized in milk composition. The return of this phosphorus to the blood is ascribed by Meigs to be the cause of higher inorganic phosphorus in mammary blood than in systemic blood.

If milk secretion proceeds at a uniform rate the ratio of phosphorus in mammary blood to that of systemic blood should be fairly constant, high in inorganic phosphorus and low in lipid phosphorus as Meigs found it. If milk secretion is at a stand-still just before milking the phosphorus content of mammary blood should be high. The lipid phosphorus should be high because it is not drawn upon; the inorganic phosphorus should

be high as Meigs suggests because of reabsorption. Our data would seem to indicate this. After milking when the alveolar cells are empty we should expect rapid absorption of milk precursors from the mammary blood. Lipoid phosphorus should therefore be lower and our analyses seem so to indicate; inorganic phosphorus should be higher but our results indicate that it is lower. If we consider, however, that depleted cells are apt to be very active cells, and a strong osmotic current must be flowing to them from the blood, then it is probable that the inorganic surplus would be retained in the alveolar cells until osmotic equilibrium should be sufficiently established to set up a counter current.

We offer the above data as incomplete evidence tending to support the idea that the rate of milk secretion is not uniform. Much more data are needed on the effect of massaging and on the variation of blood composition at intervals during a twenty-four hour period. Analyses of mammary blood before and after milking should include other precursors such as amino acids and blood sugar. The effect of pressure on milk secretion needs further investigation.

#### CONCLUSION

With few exceptions our data show that mammary blood just before milking is higher both in inorganic and organic phosphorus than systemic blood. Just after milking both forms of phosphorus are lower in mammary blood. We interpret these results to mean that just before milking synthesis of milk is practically nil, which allows the blood to retain its load of lipoids. At the same time inorganic phosphorus liberated during the hours of active secretion is being absorbed by the blood.

Just after milking both forms of phosphorus in the mammary blood are reduced. The lipoids are being used for milk fat synthesis and the inorganic phosphorus liberated by the process is not at once reabsorbed by the blood because the main osmotic current is toward the alveolar cells. During milking or four to six hours later lipoids are being utilized. After four to six hours the inorganic phosphorus in the mammary blood increases

probably because the osmotic interchange is sufficiently equalized to allow its return.

From evidence found in the literature supported by our observations and analyses, we believe that milk secretion is most active just after milking and that it proceeds in diminishing rate as the pressure and presence of accumulating synthesized milk interferes with cellular activity. The pressure of a well filled udder is probably due for the most part to intra-cellular milk vacuoles rather than from surcharged cisterns, ducts and lumina. The liberation of this vacuole milk by the stimulus of milking doubtless gave rise to the old idea that milk is secreted mainly during the milking process.

#### REFERENCES

- (1) MARSHALL, F. H. A.: *Physiology of Reproduction*. Longmans & Company, 1922.
- (2) JUDKINS, H. F.: *The Principles of Dairying*. Wiley & Sons. 1924.
- (3) GAINES, W. L.: A contribution to the physiology of lactation. *Amer. Jour. Physiol.*, 1915, xxxviii, 303.
- (4) SWETT, W. W.: Relation of conformation and anatomy of the dairy cow to her milk and butterfat producing capacity. *Jour. Dairy Sci.*, January, 1927, x.
- (5) FOA, C.: Sul' Origine del Lattosio, Della Caseina e del Grasso del Latte. *Arch. Fisiol.*, 1912, x, 402.
- (6) ROEHRIG, A.: Experimentelle Untersuchungen über die Physiologie der Milchabsonderung. *Virchow's Arch. path. Anat.*, 1876, lxxvii, 119.
- (7) GAINES, W. L. AND SANMANN, F. P.: The quantity of milk present in the udder of the cow at milking time. *Amer. Jour. Physiol.*, 1927, lxxx, no. 3, 691.
- (8) MEIGS, E. B., BLATHERWICK, N. R., AND CARY, C. A.: Contribution to the physiology of phosphorus and calcium metabolism as related to milk secretion. *Jour. Biol. Chem.*, 1919, xxxvii.
- (9) RAGSDALE, A. C., TURNER, C. W., AND BRODY, S.: The rate of milk secretion as affected by an accumulation of milk in the mammary gland. *Jour. Dairy Sci.*, 1924, vii.
- (10) BAYLISS, W. M.: *Principles of General Physiology*. 2d ed., 1918.
- (11) JACKSON, L. C., AND ROTHERA, A. C. H.: Milk—its milk sugar, conductivity and depression of freezing point. *Biochem. Jour.*, 1914, viii.
- (12) DAVIDSON, F. A.: Experimental disturbances in the milk secretion of the cow. *Jour. Agr. Research*, 1926, xxxiii.
- (13) KAUFMANN, M., AND MAGNE, H.: Sur la consommation du glucose du Sang par la tissu de la glande mammaire. *Compt. rendu Acad.*, 1906, cxliii, 779.

- (14) CARY, C. A.: Amino acids in the blood as the precursors of milk proteins. Jour. Biol. Chem., 1920, xliii, 477.  
CARY, C. A.: The free tryptophane in the cow's blood and its utilization by the mammary gland. Proc. Amer. Soc. Biol. Chem., Jour. Biol. Chem., 1926, lxvii, p. xl.
- (15) BRIGGS, A. P.: Some applications of the colorimetric phosphate method. Jour. Biol. Chem., 1924, lix, 255.
- (16) BLOOR, W. R.: Methods for the determination of phosphoric acid in small amounts of blood. Jour. Biol. Chem., 1918, xxxvi.
- (17) PRÖSCHER, FR.: Die Beziehungen der Wachstumsgeschwindigkeit des Säuglings zur Zusammensetzung der Milch bei verschiedenen Säugethieren. Zeit. f. Physiol. Chem., 1898, 24, 285.



# THE DETERMINATION OF MOISTURE IN DRY SKIM MILK BY THE BIDWELL-STERLING TOLUENE-DISTILLATION METHOD\*

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A comparison of the results obtained from a number of moisture determinations on dry skim milk by the use of the vacuum oven and the Bidwell-Sterling (1) toluene-distillation methods shows that in all cases the percentages were greater when the latter method was used.

Dry skim milk contains on the average 48.74 per cent of lactose. If this component is assumed to be in the form of the monohydrate, the combined water is equivalent to 5 per cent of the weight of the lactose or 2.44 per cent of that of the product.

According to Gillis (2) lactose loses its water of crystallization when placed under reduced pressure and in the presence of a dehydrating agent at 70°C. for ten to fifteen hours. The author found, however, that a sample of pure lactose monohydrate heated in a vacuum oven maintained at 27 inches vacuum and at a temperature of 100°C. was still losing weight at the end of thirty-four hours.

Jones and McLachlan (3) determined the moisture in lactose hydrate by the toluene-distillation method and recovered approximately the theoretical amount after five hours' distillation. Ninety per cent of the amount was recovered after two and one-half hours' distillation. The author has confirmed these results.

A comparison of the two methods when used to determine the moisture content of two dry milks is given in figure 1, wherein are plotted the results of determinations made for various time intervals.

These results, as well as those obtained when longer time

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TABLE 1

Showing results obtained by the vacuum oven and the Bidwell-Sterling toluene distillation methods

TYPE OF DRY SKIM MILK	VACUUM OVEN METHOD*	BIDWELL-STERLING METHOD†	DIFFERENCE
	per cent	per cent	per cent
Spray.....	2.57	2.80	+0.23
	3.22	3.48	+0.26
	3.86	4.25	+0.39
	2.49	2.74	+0.25
Flake.....	2.82	3.12	+0.30
Drum dried.....	2.94	3.13	+0.19
	3.91	3.96	+0.05

\* Sample maintained at 100° C. and 27 inches vacuum for two and one-half hours.

† Distillation continued for one and one-half hours.

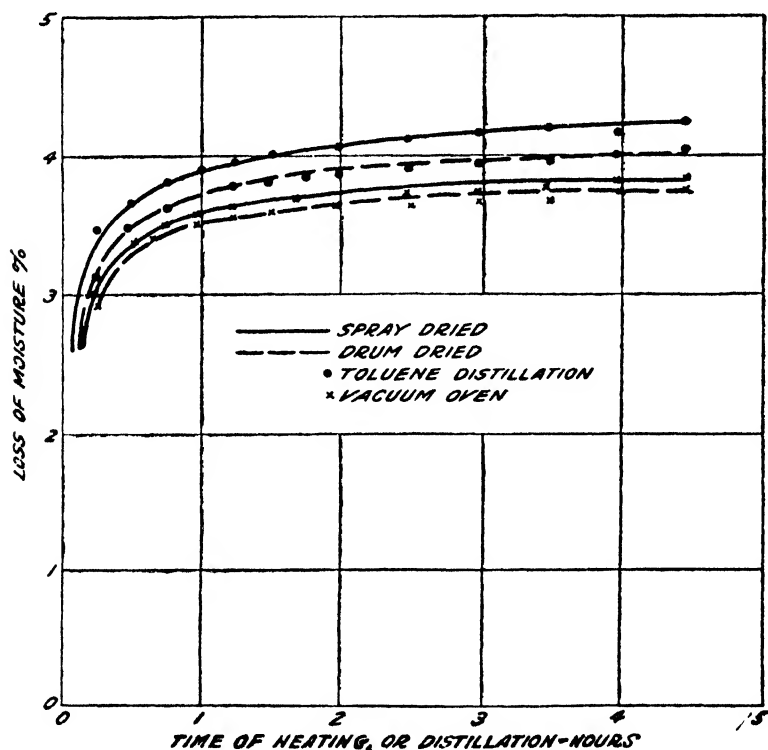


FIG. 1. SHOWING THE RATE OF LOSS OF MOISTURE OBTAINED BY THE VACUUM OVEN AND THE BIDWELL-STERLING TOLUENE-DISTILLATION METHODS

intervals were used, indicate a progressive and continuous loss of weight, thereby indicating some decomposition. This decomposition is also indicated by the yellowish color of the dry skim milk residue which with prolonged boiling becomes a brownish shade. The decomposition of lactose occurred readily in the presence of disodium phosphate.

These experiments and a number of others upon lactose and upon dry milks, indicate that of the two methods the Bidwell-Sterling toluene-distillation method furnishes the more reliable results. Because of the progressive continuous loss of weight (fig. 1) some arbitrary distillation time must be chosen. Two hours seems to furnish satisfactory results.

In addition to the greater reliability in results the Bidwell-Sterling method is more suited to commercial conditions. Vacuum pump and oven equipment are unnecessary, and since 50-gram samples are used a comparatively inexpensive balance may replace the fine chemical balance.

#### SUMMARY

Comparisons are made between the vacuum-oven method and the Bidwell-Sterling toluene-distillation method for the determination of moisture in dry skim milk.

The use of the toluene-distillation method, modified to use a 50-gram charge and a two-hour distillation time, is recommended.

#### REFERENCES

- (1) BIDWELL, G. L., AND STERLING, W. F.: Preliminary notes on the direct determination of moisture. *Ind. Eng. Chem.*, xvii, 147 (1925).
- (2) GILLIS, J.: Nouvelles recherches sur le sucre de lait. *Rec. trav. chim.*, xxxix, 88 (1920).
- (3) JONES, J. M., AND McLACHLAN, T.: The determination of moisture by the volatile solvent method. *Analyst*, lii, 383 (1927).

# THE HEAT STABILITY AND FEATHERING OF SWEET CREAM, AS AFFECTED BY DIFFERENT HOMOGENIZATION PRESSURES AND DIFFERENT TEMPERATURES OF FOREWARMING\*

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In the preparation of sweet cream for market purposes, the prevailing practice is to homogenize the product at 1000 to 1500 pounds per square inch after pasteurization at approximately 64° to 74°C. The lowest possible homogenization pressures that will prevent the separation of the fat globules under marketing conditions are used, since it is assumed that increases in the pressure greatly affect the heat stability and are therefore apt to promote "feathering."

It has been noted repeatedly in work in these laboratories that the stability of a milk of high butterfat content to heat varies greatly with the temperature of forewarming and that the forewarming temperature producing maximum stability is not the temperature generally used for pasteurization.

The changes in stability to heat which were found to occur in sweet cream under varying conditions of homogenization and temperatures of forewarming, and their relation to "feathering" and to the preparation of sterile cream are considered in this paper.

## EXPERIMENTAL

### *Basic relationships*

The relationships which exist between the temperatures and duration of heating of cream before homogenization at various pressures, the percentage of butterfat in the cream, and the time of coagulation of the product have been determined.

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TABLE 1

*Relationship of time of coagulation to butter fat content, homogenization pressure and preheating temperature of milks and creams*

BUTTERFAT CONTENT	HOMOGENI- SATION PRESSURE	TIME OF COAGULATION AT 136°C.				
		Check, not heated, not homogenised	Preheating temperature			
			60°C.	70°C.	80°C.	90°C.
<i>per cent</i>	<i>pounds per square inch</i>	<i>minutes</i>	<i>minutes</i>	<i>minutes</i>	<i>minutes</i>	<i>minutes</i>
0	1,000	150	151	148	145	146
3	2,500	124	100	95	107	119
4	2,500	98	80	84	98	102
5	500	132	131	129	131	134
	750	132	131	129	131	134
	1,000	110	85	82	85	93
	2,000	137	101	114	122	120
	2,500	137	104	116	126	117
	3,000	137	97	118	129	115
6	2,500	134	63	70	104	107
8	2,500	98	67	77	90	83
10	1,000	107	81	72	100	
	1,000	120	82	79	95	110
	2,000	107	60		100	
	2,500	120	66	82	100	83
	3,000	107	60		97	
	3,500	120	66	87	100	82
13	1,000	120	60	70	90	90
20	500	120	74	95	113	100
	750	120	50	62	112	105
	1,000	133	7	12	85	60
	1,000	123	20	80	117	120
	1,000	115	5	34	86	63
	1,500	110	2	10	75	62
	2,000	115	1	37	70	30
	2,000	110	0		70	
	2,500	115 (?)	-0	25	58	45
	2,500	115	1	40	60	17
	3,000	115 (?)	-0	35	58	40
	3,500	115 (?)	0	40	59	28
	3,500	115	2	48	46	0

TABLE 1—Concluded

BUTTERFAT CONTENT	HOMOGENI- ZATION PRESSURE	TIME OF COAGULATION AT 120°C.				
		Check, not heated, not homogenized	Preheating temperature			
			60°C.	70°C.	80°C.	90°C.
per cent	pounds per square inch	minutes	minutes	minutes	minutes	minutes
30	500	140 (?)	2	65	85	74
	500	137	—0	10	97	71
	750	140 (?)	0	42	85	0
	1,000	140 (?)	0	0	1	1
	1,000	137	—0	1	72	61
	2,000	137	—0	—0	—0	—0

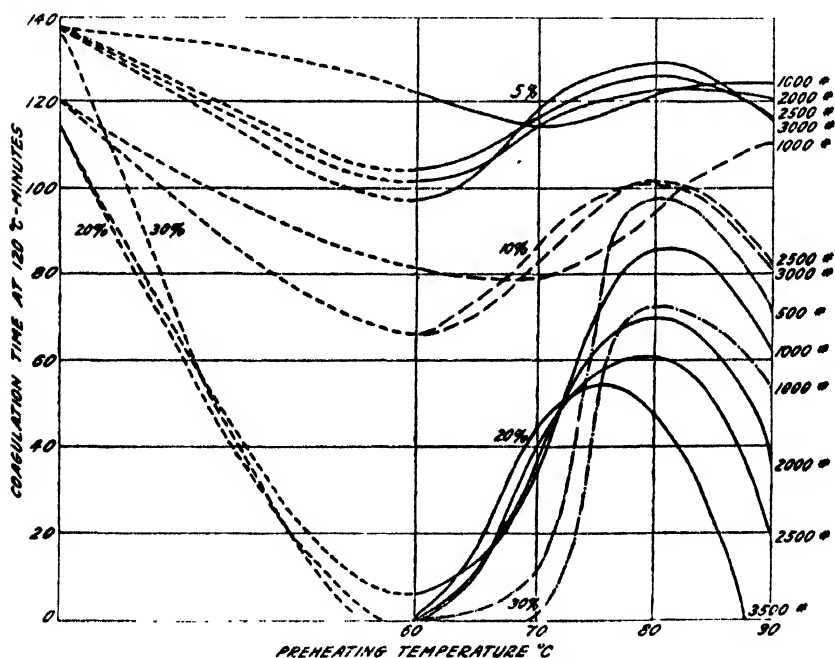


FIG. 1. RELATIONSHIPS OF BUTTERFAT CONTENT, HOMOGENIZATION PRESSURE AND TEMPERATURE OF PREHEATING TO THE HEAT STABILITY OF MILKS AND CREAMS

The method of procedure was as follows: The cream was first heated to the required temperature after which, in most cases, it was immediately homogenized and the coagulation time determined by sterilization at 120°C. In this paper the term "pre-heating temperature" is used to designate that temperature to which the cream was heated without holding, before sterilization. The homogenizer used was a 90-gallon, Progress type. To find the coagulation time, samples were sterilized in tins at 120°C. until the first signs of coagulation were noticed. The

TABLE 2

*Showing the effect of the duration of heating upon the heat stability of cream containing 80 per cent butterfat*

TIME OF COAGULATION AT 120°C.												
HOMOGENI- ZATION PRESSURE	Sample A—acidity 0.145 per cent, pH 6.68				Sample B—acidity 0.165 per cent, pH 6.63							
	Preheated		Forewarmed 10 minutes		Preheated				Pasteurized 80 minutes			
	60°	80°	60°	80°	62°	70°	80°	90°	62°	70°	80°	90°
pounds per square inch	minutes	minutes	minutes	minutes	minutes	minutes	minutes	minutes	minutes	minutes	minutes	minutes
1,000	5	86	3	70	8		61		4	58	60	35
2,000	1	70	1	44	—0		47		—0	40	25	0
2,500	1	60	1	38	0		49		—0	44	17	—0

limit of experimental error in determining this time of coagulation was approximately  $\pm 1$  minute.

The creams used were separated from fresh milks and standardized to the desired butterfat contents with skim milk. The resulting products were of excellent quality, the titratable acidities being generally less than 0.15 per cent lactic acid and of H-ion concentrations of from pH 6.70 to 6.60.

The results in table 1 show the relationship between butterfat content, homogenization pressure, and preheating temperature to the heat stability of milks and creams.

In figure 1 are plotted a few of these results.

In the manufacture of evaporated milk the time of forewarming

of the milk is of some importance and an increase in this factor, to a certain limit, increases the heat stability of the final product.

The effect of holding cream of 20 per cent butterfat content at the preheating temperatures before homogenization is given in table 2.

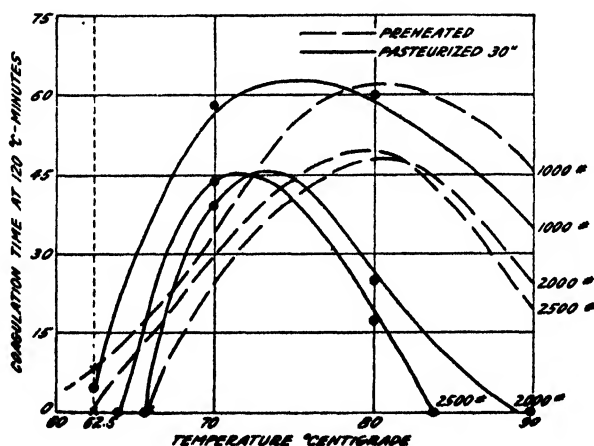


FIG. 2. EFFECT OF DURATION OF HEATING UPON HEAT STABILITY OF CREAM CONTAINING 20 PER CENT BUTTERFAT

TABLE 3

Showing the coagulation time in minutes at 120°C. of creams varying in the order of preheating and homogenization

BUTTERFAT CONTENT	HOMOGENIZATION PRESSURE	HEAT TREATMENT	CHICK	60°	70°	80°	90°
per cent	pounds per square inch		min-utes	min-utes	min-utes	min-utes	min-utes
20	1,000	Preheated before homogenization	125	20	80	117	120
		Preheated after homogenization at 60°	125	32	32	30	21

These data indicate that the figures obtained upon preheated cream (table 1, fig. 1) also hold true for pasteurized cream with the modification that as the time of holding increases the maximum of the stability curve shifts from 80°C. toward 70°C., the extent depending upon the duration of the heating period and



slightly upon the homogenization pressure. This is well illustrated in figure 2 which is plotted from the data in tables 1 and 2.

*Homogenization prior to preheating and at different temperatures*

Results of experiments wherein homogenization at 60°C. is carried out prior to preheating show clearly that preheating must be carried out prior to homogenization. Some of the results obtained are given in table 3.

Homogenization at the preheating temperatures or at 60°C. subsequent to preheating to the various temperatures yields values that differ but slightly from those obtained when the

TABLE 4

*Showing the coagulation time in minutes at 120°C. of creams homogenized at 60°C. or at the preheating temperatures*

BUTTERFAT CONTENT	HOMOGENI- ZATION PRESSURE	PREHEATED, NOT COOLED BEFORE HOMOGENIZATION			PREHEATED BUT COOLED TO 60° BEFORE HOMO- GENIZATION	
		60°	80°	90°	80°	90°
<i>per cent</i>	<i>pounds per square inch</i>	<i>minutes</i>	<i>minutes</i>	<i>minutes</i>	<i>minutes</i>	<i>minutes</i>
10	1,000	82	95	110	97	95
	2,500	66	100	83	95	81
	3,000	66	100	82	95	79

homogenization is carried out at the various preheating temperatures subsequent to the heating (table 4).

*Viscosity and heat stability*

A strikingly consistent relationship was found to exist between the heat stability and the viscosity of the product. The viscosities were determined by an Ostwald capillary pipette and are expressed as relative viscosities (water = 1). The results are shown in table 5.

These data are plotted in figure 3 which shows the reciprocal relationship existing between the viscosity and the heat stability.

*Feathering of cream*

The formation of a flocculent coagulum, known as feathering, when sweet cream is added to hot coffee, is a problem of con-

TABLE 5

*Showing the relative viscosity and time of coagulation of various creams preheated and homogenized at various temperatures and pressures, respectively*

HOMOGENI- ZATION PRESSURE	TEMPER- ATURE OF PREHEAT- ING	5 PER CENT BUTTER FAT		10 PER CENT BUTTERFAT		20 PER CENT BUTTERFAT		30 PER CENT BUTTERFAT	
		Time of coagu- lation at 120°C.	Rela- tive vis- cosity*	Time of coagu- lation at 120°C.	Rela- tive vis- cosity*	Time of coagu- lation at 120°C.	Relative viscosity*	Time of coagu- lation at 120°C.	Relative viscosity*
pounds per square inch	°C.	minutes		minutes		minutes		minutes	
1,000	60	122	1.35	82	1.81	5	1.75	—0	4.18
	70	114	1.31	79	1.70	34	1.58	1	4.91
	80	121	1.47	95	1.72	86	1.49	72	2.24
	90	124	1.50	110	1.78	63	1.58	61	3.30
2,000	60	101	1.45			1	3.50	—0	Very viscous
	70	114	1.47			37	1.84	—0	13.80
	80	122	1.47			70	1.58	—0	5.34
	90	120	1.52			30	2.02	—0	Very viscous
2,500	60	104	1.38	66	2.18	1	4.05		
	70	116	1.37	82	1.88	40	1.75		
	80	126	1.48	100	1.76	60	1.58		
	90	117	1.50	83	1.88	17	3.16		
3,000†	60	97	1.49	66	2.17	2	3.65		
	70	118	1.45	87	1.91	48	1.75		
	80	129	1.47	100	1.65	46	1.66		
	90	115	1.52	82	1.89	0	Very viscous		

\* Water = 1.

† 3500 pounds used for the 20 per cent cream.

siderable commercial importance. Burgwald (1) points out that the acidity of the product is an important factor in the feathering phenomenon.

In view of the fact that acidity is probably the controlling factor in feathering, it was deemed advisable to ascertain the true hydrogen-ion concentration as well as the titratable acidity of the samples. Accordingly the hydrogen-ion concentration

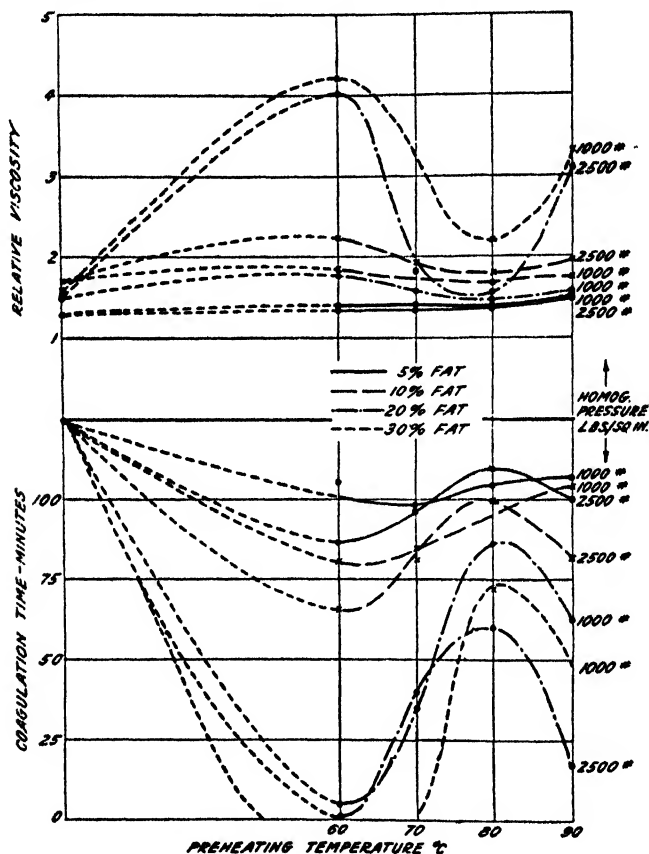


FIG. 3. SHOWING THE RECIPROCAL NATURE OF CHANGES IN HEAT STABILITY AND RELATIVE VISCOSITY

was determined by the quinhydrone electrode (2) on numerous samples of 20 per cent cream. The results of these determinations, plotted against the titratable acidity and expressed in terms of lactic acid, are shown in figure 4. The curve obtained

by Sharp and McInerney (3) for fresh milk is reproduced on the same figure.

Feathering tests were carried out in three different ways:

1. Addition of water (93° to 97°C.) to cream
2. Addition of coffee (93° to 97°C.) to cream
3. Addition of cream to coffee (86° to 90°C.)

Feathering in coffee occurs at a much lower acidity than feathering in water, due probably to the presence of tannin and

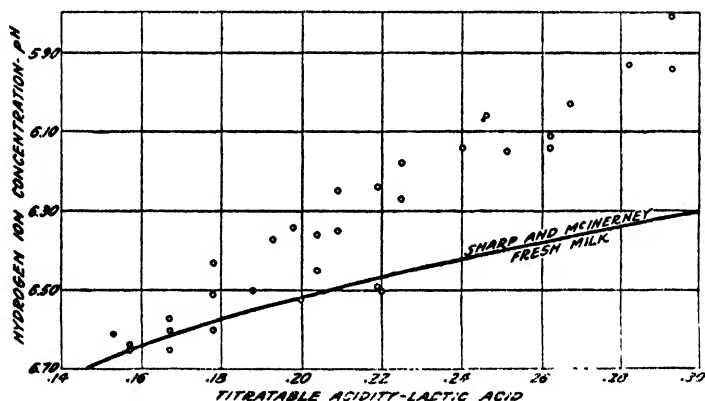


FIG. 4. RELATIONSHIP BETWEEN TITRATABLE ACIDITY AND pH OF CREAM CONTAINING 20 PER CENT BUTTERFAT

salts in the coffee which promote coagulation. As pointed out by Burgwald (1) cream will feather at a lower acidity when coffee is added to it, than when cream is added to coffee.

The results obtained on cream feathering are shown in table 6. The results in part 1 of this table are for preheated cream, and the feathering tests were conducted in water. The data in the second part of table 6 are for cream pasteurized thirty minutes, and feathering tests were here conducted in coffee. Two extremes are thus represented. The cream used to obtain the data in part 1, being subjected to conditions less severe than that of part 2, shows a higher acidity necessary before feathering

TABLE 6

*Showing the degree of feathering of cream that contains 20 per cent butterfat and that is subjected to various heating temperatures before homogenization*

## Part 1. Preheated cream

TITRATABLE ACIDITY	pH	HOMOGENI- ZATION PRESSURE	FEATHERING IN WATER (93° TO 97°C.) AT RESPECTIVE PREHEATING TEMPERATURES			
			60°	70°	80°	90°
<i>per cent</i>		<i>pounds per square inch</i>				
0.156		2,500	+		—	
0.167	6.57	2,500	+		—	
0.193	6.37	1,000-3,000	+++		—	
0.204	6.45	1,000	+	—	—	—
0.204	6.45	2,500	++	—	—	—
0.209	6.25	2,500	+++	+	—	
0.22	6.49	1,000	+	—	—	—
0.22	6.49	2,500	++	—	—	—
0.24	6.14	2,000	+++	+++	—	+
0.292		1,000-2,500	+++	+++	+++	+++

## Part 2. Pasteurized 30 minutes

TITRATABLE ACIDITY	pH	HOMOGENI- ZATION PRESSURE	CREAM ADDED TO COFFEE			COFFEE ADDED TO CREAM		
			62.5°	70°	80°	62.5°	70°	80°
<i>per cent</i>		<i>pounds per square inch</i>						
0.15 or less	6.65-6.75	1,000-2,500	—	—	—	—	—	—
0.167	6.65	1,000	—	—	+++	+++	++	+++
0.167	6.65	2,000	++	+	+++	+++	++	+++
0.188		1,500		—	++		+++	
0.188		2,500		++	+++		+++	+++
0.22	6.25	1,000-1,500	Feath- ing	++		Feath- ing	+++	
0.22	6.25	2,000	cer- tain	+++		cer- tain	+++	
0.246	6.14	1,500		+	—		+++	
0.246	6.14	2,000		+			+++	+
0.246	6.14	2,500		+			+++	

—, no feathering; +, trace of feathering; ++, feathering; +++, heavy feathering.

develops. The treatment given the cream used to obtain the results shown in part 2 corresponds to ordinary commercial practice.

A study of part 1 of table 6 shows that the tendency of cream to feather follows closely the tendency of cream to coagulate upon sterilization according to the figures in table 1. The data in figure 1 may therefore be considered as representing the relative tendency of cream to feather under the various conditions of preheating, homogenization pressure, and butterfat percentages given.

The results given in table 2 indicate that when cream is pasteurized instead of preheated the point of maximum stability shifts from 80°C. toward 70°C. It would appear, therefore, that cream to be homogenized for market purposes should be pasteurized at approximately 70° to 74° for thirty minutes. This conclusion is further borne out by the fact that (table 6, part 2) cream pasteurized at 70°C. was generally less susceptible to feathering than those samples pasteurized at 62.5° or 80°C.

Knowledge of the acidity of the cream to be homogenized is the best criterion of the amount of pressure which the product will withstand without feathering. An acidity of 0.165 per cent would appear to mark the danger point above which homogenized cream very probably will feather. A titratable acidity of 0.165 per cent will generally allow a cream containing 20 per cent butter fat to be pasteurized at 70° to 74°C. and homogenized at 1000 to 1500 pounds pressure without disastrous effects. It must further be remembered that the temperature of the coffee used in the test greatly affects feathering. A coagulation will occur in coffee at boiling temperature much more readily than in coffee cool enough to drink.

#### DISCUSSION

It is evident that at every pressure of homogenization and for any percentage of butterfat, as high as 30 per cent, the maximum heat stability is attained by preheating the cream to 80°C., excepting for those of low fat content and at low homogenization pressures when 90°C. slightly increases the stability. At 60°C., the lowest temperature at which it is practical to homogenize, the minimum heat stability of all creams is reached except for those of very low butterfat content for which the heat stability curve sometimes reaches its lowest point at 70°C.

Pasteurization of 20-per-cent cream for thirty minutes causes the maximum of the heat stability curve to shift from 80°C. toward 70°C.

Feathering, being a process of heat coagulation, follows the heat stability curve, and consequently pasteurization of coffee cream is most advantageously carried out at 70° to 74°C. (158° to 165°F.). This practice is, at present, widely used among commercial plants as a safeguard against bacterial action. It now appears also to be the best temperature to use to prevent feathering.

The temperature of pasteurization and the homogenization pressures are, however, minor factors to be considered when compared with the quality of the cream. In no case during this work was cream which contained 20 per cent butterfat and which was preheated at any temperature from 60° to 90° and homogenized at any pressure up to 3000 pounds found to feather if the cream used was strictly fresh and of low acidity. Occasionally, however, creams, abnormal in some respects, may feather, even though they be of apparent excellent quality.

The problem of feathering is, then, mainly a problem of quality of product. Favorable heat treatment and homogenization pressure may aid in preventing feathering in a poor lot of cream but the sure way to overcome the difficulty is the use of only the highest grade of cream with a titratable acidity below 0.15 per cent.

The quality of a cream in this respect may be judged by acidity or H-ion concentration determinations. Either test is sufficient for determining approximately the heat treatment and homogenization pressure which a cream will withstand.

Because of variations in chemical or physical properties in different creams no definite limits can be expressed for the acidity danger line, with respect to feathering. The data given, therefore, should be interpreted as setting forth the principles governing feathering and not the solution of this problem for each case.

The interesting fact that such a wide difference exists in the heat stability of sweet cream when either the butterfat percentage, heating temperature, or homogenization pressure is varied, presents an extensive field for speculation as to the probable chemical and physical changes which take place in the product.

The reciprocal relationship existing between the heat stability curve and the viscosity curve for a given cream, as shown in figure 2, may be significant. The cause of increased viscosity in homogenized cream is generally considered to be due to a clumping of the fat globules.

Doan (4) has been able to distinguish viscolized milk by the characteristic clumps of its fat globules due to viscolization.

It is well known that a change in the salt equilibrium and the formation of acid during heating may greatly affect heat coagulation. It is possible, therefore, only to suggest at this time that the degree of clumping is a result of variation in potential of the fat globules and that this is an important factor in the heat stability of homogenized creams.

Considerable attention has been given in this work to the preheating of cream as contrasted with its pasteurization or forewarming for a specified time. This has been done because of the value of such results when applied to a process for the manufacture of sterile cream as developed in these laboratories (5).

A study of figure 1 has shown that for maximum stability sweet cream containing 20 per cent butterfat and of good quality should be preheated to 80°C. before its homogenization instead of to the lower temperatures customarily used. The subsequent sterilization makes previous pasteurization or forewarming unnecessary.

The troublesome heat coagulation encountered in the sterilization of homogenized cream can accordingly be eliminated by preheating the product to 80°C. before homogenization. The fact must be recognized, however, that any homogenized cream, to withstand ten to fifteen minutes sterilization, must originally be of low acidity and good quality. The curves in figure 1 for cream containing 20 per cent butterfat show the relative margin of safety which exists between the various homogenization pressures when an 80°C. preheating temperature is used. Average cream of over 0.15 per cent titratable acidity would probably coagulate in less than half the time which was found for the product used here.



## SUMMARY

1. The heat stability of cream, measured in terms of time of coagulation at 120°C. as affected by butterfat content, temperature of heating before homogenization, and homogenization pressure has been determined. A definite relationship was found in their influence on heat stability.

2. An increase of homogenization pressure lowered the time of coagulation. Maximum stability resulting from preheating occurred when 80°C. was used, and minimum stability during sterilization occurred when 60°C. was used. An increase in butterfat content invariably lowered the heat stability of homogenized cream.

3. The same general relationship exists in pasteurized as in preheated cream, with the exception that the point of maximum heat stability shifts from 80°C. in preheated creams to approximately 70° to 74°C. in pasteurized creams.

4. Where pasteurization was practiced prevention of feathering was partially successful by the treatment producing maximum heat stability, namely pasteurization for thirty minutes at 70° to 74°C.

5. The basic criterion for predicting whether or not a cream will feather is quality. Acidity and H-ion concentration were used for quality determinations. Pure, fresh cream of 20 per cent butterfat content and of 0.15 per cent acidity can generally be pasteurized from 65° to 85°C. and homogenized at any pressure up to 3000 pounds without danger of feathering. With optimum treatment 0.165 per cent acidity is the danger line for feathering.

6. An increase of viscosity of preheated homogenized cream is accompanied by a decrease in heat stability. It is suggested that variation of potential upon the fat globules may be an important factor in determining the heat stability of the product.

7. In the preparation of sterile cream for market purposes maximum stability during sterilization was obtained by preheating to approximately 80°C. before homogenization.

## REFERENCES

- (1) BURGWARD, L. H.: Some factors which influence the feathering of cream in coffee. Jour. Agr. Research, xxvi, no. 11, 1923.
- (2) WATSON, P. D.: Use of the quinhydrone electrode for following changes in pH in Swiss cheese. Ind. Eng. Chem., xix, 1927, p. 1272.
- (3) SHARP, P. F., AND MCINERNEY, T. J.: Relation of the hydrogen-ion concentration to the titratable acidity of milk. Jour. Biol. Chem., lxxv, no. 1, 1927.
- (4) DOAN, F. L.: Viscolized milk and its detection. Jour. Dairy Sci., x, no. 6, 1927.
- (5) WEBB, B. H.: A process for sterilizing cream. U. S. Patent No. 1,646,671.



# PHYSICAL CONSTANTS OF THE MILK AS INFLUENCING THE CENTRIFUGAL SEPARATION OF CREAM AT VARIOUS TEMPERATURES\*

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Studies by Rahn (4) van der Burg (1), (2) van Dam and Sirks (3) and Troy and Sharp (5) have shown that Stokes equation indicates quite accurately the rate of rise of individual fat globules through milk plasma under the influence of gravity. Troy and Sharp (5) have also shown that the rate of rise of clusters of fat globules through milk plasma is in agreement with this equation. Stokes equation is as follows:

$$V = \frac{2 r^2 (d_p - d_f) a}{9 \eta} \quad (1)$$

Where  $V$  is the rate of movement of the fat globule in centimeters per second,  $r$  the radius of the fat globule,  $\eta$  the viscosity of the milk plasma,  $d_p$  and  $d_f$  the density of the plasma and fat respectively, and  $a$  is the acceleration. In the case of fat globules rising under the influence of gravity,  $a$  is the gravitational constant and is numerically equal to 980 dynes.

This equation can also be used to calculate the velocity of the movement of fat globules through the plasma due to the centrifugal force of the cream separator, by expressing  $a$  in terms of the acceleration produced by the centrifuge. Equation (2) gives the value of  $a$ , when the acceleration is due to centrifugal force.

$$a = \frac{(2 \pi n)^2 R}{(60)^2} \quad (2)$$

Where  $n$  is the number of revolutions of the separator bowl per minute, and  $R$  is the distance of the fat globule from the axis of rotation. Thus the rate of movement of a fat globule through

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the milk plasma due to the centrifugal force at any instant, is given by the following equation:

$$V = \frac{2 r^2 (d_p - d_f) 4 \pi^2 n^2 R}{9 \eta 3600} \quad (3)$$

Since several of the factors in the equation are numbers they may be gathered together into a numerical constant which has the value 0.00244. The equation then becomes:

$$V = \frac{0.00244 (d_p - d_f) r^2 n^2 R}{\eta} \quad (4)$$

Equation (4) is of general applicability. In order to apply the equation to the separation of fat from milk, all of the factors in equation (4) which are independent of the dimensions and speed of the separator and the size of the fat globules were gathered into a constant  $K$ , which varies with the temperature.

$$K = \frac{0.00244 (d_p - d_f)}{\eta} \quad (5)$$

The velocity of movement then is given by the equation:

$$V = K r^2 R n^2 \quad (6)$$

For a given cream separator, if the speed of the bowl and rate of flow of the milk through the separator are held constant, then the rate of movement of the fat globules through the plasma, and consequently the effective force tending to separate the fat, depends on the value of  $K$ , which in turn is controlled by the densities of the plasma and fat and by the viscosity of the plasma. A glance at the equations shows that the separation would be more complete the greater the difference in density between the fat and the plasma, and the lower the viscosity.

Since temperature markedly affects the density of the fat and the viscosity of the plasma, the values for  $K$  were calculated for 5°C. intervals of temperature from 5° to 80°C. to show how the effective force tending to separate the cream in a given separator increases with temperature. The results are given in table 1.

The data in columns (2) and (5) are taken from the paper by Whitaker, Sherman and Sharp (6). The density of the fat, column (3), was determined experimentally with pycnometers. The value of  $K$  for the various temperatures is given in column (6).

The density and viscosity both change in such a way as to make the separation more efficient at the higher temperatures.

TABLE 1

*Density of plasma and fat, and the viscosity of the plasma as affecting the velocity of movement of the fat globules under centrifugal force*

TEMPER- ATURE	DENSITY OF PLASMA $d_s$	DENSITY OF FAT $d_f$	DIFFER- ENCE $d_s - d_f$	VISCOSITY	$K$	$K^1$	PERCENTAGE INCREASE IN $K^1$ FOR EACH 5°C. INTERVAL INCREASE IN TEMPER- ATURE
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
°C.				poise			per cent
5	1.0365	0.9612	0.0753	0.0296	0.0062	0.0062	
10	1.0359	0.9528	0.0831	0.0247	0.0082	0.0083	33.0
15	1.0348	0.9421	0.0927	0.0210	0.0108	0.0110	33.0
20	1.0338	0.9304	0.1034	0.0179	0.0141	0.0144	30.9
25	1.0322	0.9208	0.1114	0.0154	0.0176	0.0181	25.9
30	1.0306	0.9119	0.1187	0.0133	0.0218	0.0226	24.9
35	1.0288	0.9082	0.1206	0.0117	0.0252	0.0262	15.9
40	1.0266	0.9050	0.1216	0.0104	0.0285	0.0297	13.4
45	1.0245	0.9012	0.1233	0.0093	0.0323	0.0337	11.3
50	1.0223	0.8982	0.1241	0.0085	0.0356	0.0372	10.4
55	1.0198	0.8945	0.1253	0.0077	0.0396	0.0415	11.4
60	1.0171	0.8913	0.1258	0.0071	0.0432	0.0454	9.1
65	1.0145	0.8881	0.1264	0.0066	0.0467	0.0492	8.4
70	1.0117	0.8848	0.1269	0.0062	0.0499	0.0527	7.1
75	1.0086	0.8813	0.1273	0.0059	0.0526	0.0558	5.9
80	1.0054	0.8778	0.1276	0.0057	0.0546	0.0580	3.9

Column (4) shows that the difference in density between the skim-milk and the fat increases rather rapidly from 5° to 35°C., but from 35° to 80°C. the increase in this difference is not so marked. Column (5) shows that the viscosity decreases rather rapidly as the temperature increases up to 35° to 40°C. Thus these two factors cause the effective force, tending to separate the fat from the plasma, to increase rather rapidly with temperature up to about

35° to 40°C. but from there on the increase with temperature is not so pronounced. Still another factor which tends to make the efficiency of separation increase more rapidly with temperature

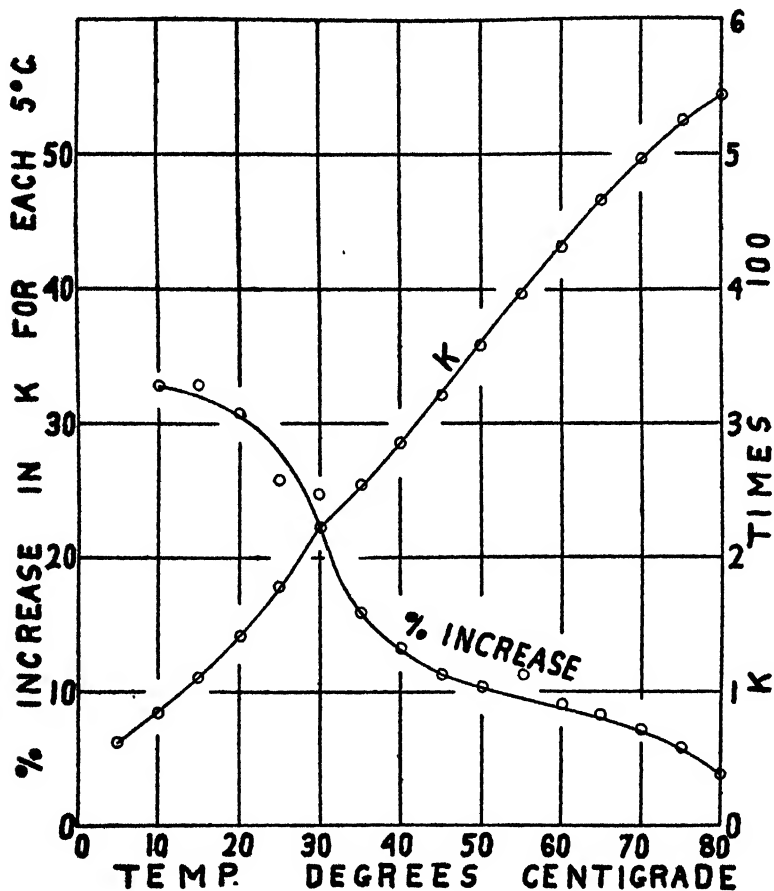


FIG. 1. INCREASE IN THE EFFECTIVE FORCE OF SEPARATION WITH TEMPERATURE AS REPRESENTED BY K, AND THE PERCENTAGE INCREASE FOR EACH 5°C. INCREMENT OF INCREASE IN TEMPERATURE

up to 35° to 40°C. is the actual expansion of the fat globules. If the size of the fat globules is measured at 5°C. their volume will be 6.2 per cent greater at 40°C. while they will increase in volume only about 3 per cent in going from 40° to 80°C. It was assumed

that the fat globules were measured at 5°C. and the effect of the increase in size of the fat globules with the increase in temperature as influencing  $K$  is given in column (7) as  $K^1$ .

The percentage increase in the constant  $K^1$  of column (7) was calculated for each 5° increment of increase in temperature. The results are given in column (8). By increasing the temperature of separation from 5° to 10°C. the effective force tending to separate the fat from the skimmilk is increased 33 per cent. On the other hand by increasing the temperature from 40° to 45°C. the increase in force tending to separate the cream is increased only about 11 per cent. The relation between temperature and the values of  $K^1$  is shown in figure 1. It is seen that the curve for the constant  $K^1$  has two parts, one in which the fat is mostly solid, at the point which the fat becomes liquid, the curve breaks to a less steep slope. The effect of temperature is shown more definitely by plotting the percentage increase in  $K^1$  for each 5°C. interval increase in temperature. Each 5°C. increase in temperature produces a marked increase in the effective force tending to separate the cream up to about 25°C. where there is a distinct break in the curve and at 35° to 45°C. the curve breaks again and tends to run more nearly horizontal. This curve shows that the effectiveness of the centrifugal force exerted, increases most markedly as to the temperature increases up to about 40°C. By increasing the temperature from 5° to 40°C. the effective force tending to separate the cream increases 380 per cent while in going from 40° to 80°C. the effective force increases only 95 per cent.

While many factors play a part in determining the temperature of cream separation, it is interesting to note that up to the temperature of 35° to 45°C. (95° to 113°F.) there is a marked increase in effective centrifugal force tending to separate the fat from the skimmilk while above this temperature the increase is not so great. Perhaps the relationship pointed out here is one of the reasons for the selection of a separation temperature near 40°C. when a recovery of the fat is the object of the separation.

There is a growing tendency to separate milk at lower and lower temperatures in order to obtain cream with a greater body. The results in table 1 indicate that if the temperature of separa-



tion is decreased from 40°C. (104°F.) to 25°C. (77°F.) a decrease of about 39 per cent in the effective force tending to separate the cream will occur.

#### SUMMARY

The effective centrifugal force tending to separate the fat from the skimmilk in a cream separator, operated at constant speed and rate of flow of milk, increases markedly as the temperature of the milk is increased up to 35° to 45°C. (95° to 113°F.), above this temperature the increase is much less pronounced.

This increase in effective force is due to the increase in the difference in density between the fat and the plasma, and to the decrease in viscosity of the plasma.

#### REFERENCES

- (1) VAN DER BURG, B. 1921 De beweging van een vetbolletje in de melk. Het algemeen Zuivelblad., January 7 (1921), p. 3-4.
- (2) VAN DER BURG, B. 1927 Etude du lait considéré comme émulsion. Le processus de la montée de la crème. Le Lait., vii, 452-466.
- (3) VAN DAM, W., AND SIRKS, H. A. 1922 Onderzoekingen over de oprooming volgens het Froesch systeem. Vers. landb. onderzoek. Rijkslandbouw., No. 26, 106-186.
- (4) RAHN, O. 1921 Untersuchungen über die Rahmbildung. Forsch. geb. Milchwirtsch. u. Molkereiwesen., i, 133-154, 165-181, 213-233.
- (5) TROY, H. C., AND SHARP, P. F. 1928 Physical factors influencing the formation and fat content of gravity cream. Jour. Dairy Sci., xi, 189-230.
- (6) WHITAKER, R., SHERMAN, J. M., AND SHARP, P. F. 1927 Effect of temperature on the viscosity of skimmilk. Jour. Dairy Sci., x, 361-371.

# GROWTH IN WEIGHT OF GUERNSEY COWS AFTER THE AGE OF TWO YEARS\*

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The growth of lactating Jersey cattle, as indicated by changes in live weight, was reported in a previous communication (1). It was found that after the age of two years the course of growth of the Jersey cow was noncyclic and the rate of decline in growth was exponential, i.e., the percentage decline in the rate of growth, as measured by weight, in unit time was constant.

The present paper is for the purpose of presenting data covering the same period in the growth of a second breed of dairy cattle, namely, the Guernsey, and to show that the characteristics of the growth curve are essentially the same as those of the Jersey breed.

In table 1 will be found the frequency distribution of the animals included in the study. Included in the table are all Advanced Registry Guernsey cows for which weight data are available. While the number is limited it is believed to be a representative population of such animals. It will be noted that the calculated normal frequency is in good agreement with the observed frequencies.

As the weight data on the cows were sent to the Guernsey Cattle Club at the close of the year's production, the weights either actual or carefully estimated were taken at the close of the lactation period. The age of the animals in this case is the age at the time the weights were taken rather than the age at commencement of test given in the previous paper. For purposes of comparison with the Jersey growth curve, a correction of one year should be made.

The statistical constants derived from table 1 are presented in table 2. The mean observed weights at half-year intervals with their probable errors may be noted. In figure 1, is shown the

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TABLE 1  
Table of age and weight of Guernsey cattle

WEIGHT CLASSES	AGE CLASSES, YEARS AT CLOSE OF LACTATION PERIOD																								TOTAL	CALCULATED NORMAL PER- CENTAGE OF LACTATION
	2 to 2.5	2.5 to 3.0	3.0 to 3.5	3.5 to 4.0	4.0 to 4.5	4.5 to 5.0	5.0 to 5.5	5.5 to 6.0	6.0 to 6.5	6.5 to 7.0	7.0 to 7.5	7.5 to 8.0	8.0 to 8.5	8.5 to 9.0	9.0 to 9.5	9.5 to 10.0	10.0 to 10.5	10.5 to 11.0	11.0 to 11.5	11.5 to 12.0	12.0 to 12.5	12.5 to 13.0	13.0 to 13.5	13.5 to 14.0		
600- 649			1	1															1						2	
650- 699	1	1	1	0	1									1					0						6	
700- 749		1	9	3	1	4	1	1					1						0						21	
750- 799		1	15	4	2	1	1	2					1						0						27	
800- 849		5	44	17	6	4	3	1	2	4			1						0						88	
850- 899		7	50	31	9	5	4	3	3	3	2		1	1	1				0						121	
900- 949		11	116	51	32	23	15	13	4	5	3	4	6	4	2	3	3	1	1						285	
950- 999		12	133	60	35	25	11	15	11	9	4	7	6	5	3	3	1	0	4	1			1	0	346	
1,000-1,049		13	155	70	53	35	36	23	19	16	13	13	4	3	4	1	2	3	0	1			0	0	404	
1,050-1,099		6	100	57	40	37	25	19	20	9	6	10	4	3	2	7	3	3	1	1		2	2	2	359	
1,100-1,149		3	85	64	41	33	33	24	22	26	18	9	10	8	3	4	6	4	2	1		2	1	0	399	
1,150-1,199		3	31	20	23	24	24	18	18	11	9	7	5	5	1	4	5	0	1	1		0	1	1	212	
1,200-1,249			19	25	17	19	29	15	15	16	12	10	5	14	2	3	4	0	1	0		3	1		212	
1,250-1,299			8	4	10	11	7	12	7	6	7	6	1	1	3	3		1	1	0		2	1		92	
1,300-1,349			7	7	11	2	4	4	2	7	2	1	2	2	1	1		2	0	0					55	
1,350-1,399			0	1	0	2	1	3	1	2	3	2	1	2	1	1		0	0	1					20	
1,400-1,449			1		2	1	3	1	2	1	2	1	0	0		0		1							15	
1,450-1,499								0	0	1		0	0		1										2	
1,500-1,549								1	1			1	1												6	
	1	63	775	415	282	228	197	156	127	116	81	71	46	51	24	31	21	15	11	7	9	7	4	3	2,741	

course of growth after two years until maturity. The smoothed curve was computed from the exponential equation

$$W_t = A (1 - e^{-kt}) \quad (1)$$

where  $W_t$  is the mean live weight at any age  $t$ ,  $A$  the weight of the animal at maturity,  $e$  the base of natural logarithms, and  $k$

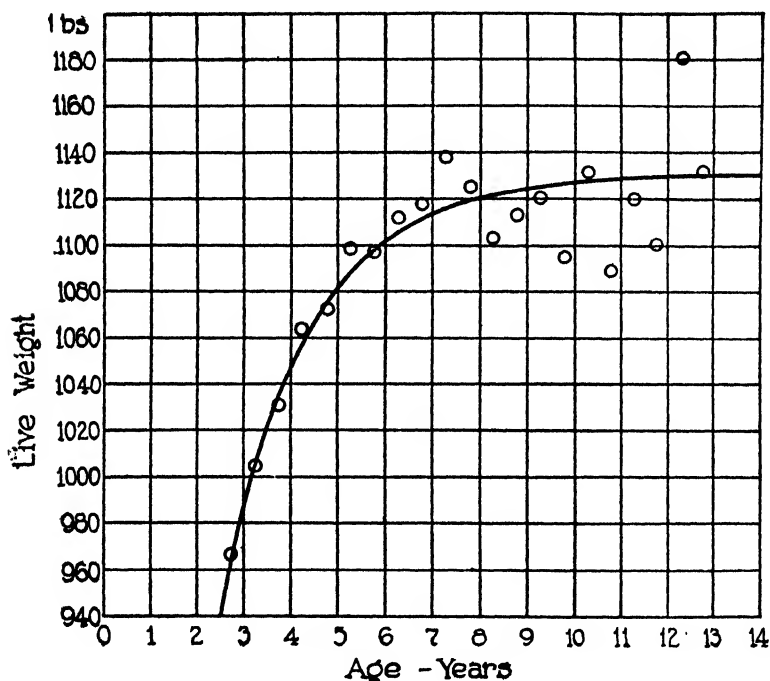


FIG. 1. RELATION BETWEEN AGE AND WEIGHT OF GUERNSEY COWS AFTER THE AGE OF TWO YEARS

The circles represent the observed values; the smooth line is the fitted curve of the equation  $W_t = 1130 (1 - e^{-0.55t})$  when  $W_t$  is the mean live weight at any age  $t$ .

the parameter indicating the rate of decline of the growth limiting reaction.

When the parameters  $A$  and  $k$  are determined, the equation takes the form

$$W_t = 1130 (1 - e^{-0.55t}) \quad (2)$$

where  $t$  is the age from conception. The birth age equals ( $t + 0.77$ ) for the calf is carried in utero 0.77 years. The fit of the

TABLE 2  
*Growth of the Guernsey cow*

AGE	NUMBER OF ANIMALS INCLUDED	LIVE WEIGHT		STANDARD DEVIATION	COEFFICIENT OF VARIATION
		Computed from formula*	Mean observed weight		
<i>years</i>					
2.75	63	967.0	967.9 $\pm$ 8.83	104.0 $\pm$ 6.25	10.7 $\pm$ 0.65
3.25	775	1,006.2	1,005.3 $\pm$ 2.75	113.3 $\pm$ 1.94	11.3 $\pm$ 0.19
3.75	415	1,035.9	1,030.4 $\pm$ 4.29	129.5 $\pm$ 3.03	12.6 $\pm$ 0.29
4.25	282	1,058.6	1,064.9 $\pm$ 4.94	123.0 $\pm$ 3.49	11.6 $\pm$ 0.33
4.75	228	1,075.7	1,071.5 $\pm$ 5.81	130.0 $\pm$ 4.11	12.1 $\pm$ 0.38
5.25	197	1,088.8	1,099.4 $\pm$ 5.86	122.0 $\pm$ 4.14	11.1 $\pm$ 0.38
5.75	156	1,098.7	1,097.8 $\pm$ 7.45	138.0 $\pm$ 5.27	12.6 $\pm$ 0.48
6.25	127	1,106.2	1,112.4 $\pm$ 7.35	120.0 $\pm$ 5.20	10.8 $\pm$ 0.47
6.75	116	1,111.9	1,118.6 $\pm$ 8.36	133.5 $\pm$ 5.91	11.9 $\pm$ 0.53
7.25	81	1,116.3	1,139.2 $\pm$ 9.18	122.5 $\pm$ 6.49	10.8 $\pm$ 0.57
7.75	71	1,119.6	1,125.0 $\pm$ 10.12	126.5 $\pm$ 7.16	11.2 $\pm$ 0.64
8.25	46	1,122.1	1,103.3 $\pm$ 13.81	139.0 $\pm$ 9.76	12.6 $\pm$ 0.88
8.75	51	1,124.0	1,113.3 $\pm$ 13.96	148.0 $\pm$ 9.87	13.3 $\pm$ 0.89
9.25	24	1,125.4	1,120.8 $\pm$ 21.20	154.0 $\pm$ 14.99	13.7 $\pm$ 1.34
9.75	31	1,126.5	1,095.9 $\pm$ 15.20	125.5 $\pm$ 10.75	11.5 $\pm$ 0.98
10.25	21	1,127.4	1,132.2 $\pm$ 10.21	69.5 $\pm$ 7.22	6.1 $\pm$ 0.64
10.75	15	1,128.0	1,088.3 $\pm$ 26.94	155.0 $\pm$ 19.05	14.2 $\pm$ 1.75
11.25	11	1,128.5	1,120.5 $\pm$ 28.54	140.5 $\pm$ 20.18	12.5 $\pm$ 1.80
11.75	7	1,128.9	1,101.4 $\pm$ 35.51	139.5 $\pm$ 25.10	12.7 $\pm$ 2.28
12.25	9		1,180.6 $\pm$ 17.09	76.0 $\pm$ 12.08	6.4 $\pm$ 1.02
12.75	7		1,132.2 $\pm$ 23.93	94.0 $\pm$ 16.92	8.3 $\pm$ 1.49
13.25	4		1,062.5 $\pm$ 30.18	89.5 $\pm$ 21.34	8.4 $\pm$ 2.01
13.75	3		1,241.7 $\pm$ 9.14	23.6 $\pm$ 6.46	1.9 $\pm$ 0.52
Total.....	2,740		1,057.6 $\pm$ 1.69	131.0 $\pm$ 1.19	12.4 $\pm$ 0.11

\* Computed from the equation  $W_t = 1130 (1 - e^{-0.56t})$ .

equation is good where the data are numerous enough to be reliable.

The equation for the growth of the Jersey cow was

$$W_t = 960 (1 - e^{-0.60t})$$

It will be noted that the average mature weight of the Guernsey Advanced Registry cow is 170 pounds greater than the Jersey, whereas, the value of  $k$  is 0.05 less. The difference in the latter indicates that the rate of growth of the Jersey cow is slightly greater than the rate of growth of the Guernsey cow. However, no statistical measure of the reliability of  $k$  is available. It is possible, therefore, that the small observed difference in  $k$  may not be significant.

#### SUMMARY

Data are presented on the change of weight with age of the Guernsey cow. It was shown that from the age of first calving at about two years, until the age of maximum body weight, the course of growth in body weight can be accurately represented by an exponential equation.

It is a pleasure to acknowledge the indebtedness of the writer to Mr. C. M. Cummings of the Advanced Registry Division of the American Guernsey Cattle Club for the original data.

#### REFERENCE

- (1) TURNER, C. W., RAGSDALE, A. C., AND BRODY, S.: Jour. Dairy Sci., vi, no. 5.

# LIPINS AND STEROLS AS SOURCES OF ERROR IN THE ESTIMATION OF FAT IN BUTTERMILK BY ETHER EXTRACTION METHODS\*

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The earliest authentic method of determining the percentage fat content of dairy products was the so-called gravimetric, in which the fatty substances were extracted by means of fat solvents, an aliquot or all of the solvents evaporated, and the amount of fatty substances measured by weighing. Numerous methods of procedure were suggested, all of which were essentially the same in principle. In 1890 the development of the Babcock test (1) offered a more rapid means of estimating the fat content of milk. In the following year Myers (2) made comparisons which indicated that in testing buttermilk Babcock's method gave results considerably lower than those obtained by the Adams gravimetric. Because the gravimetric method was considered as standard, the Babcock results were looked upon as too low. Later the gravimetric procedure of Roese (3), improved by Gottlieb (4), and known as the Roese-Gottlieb method was made official by the Association of Official Agricultural Chemists. Europe has made greater use of this method than has this country, but throughout the world it has been considered to be the only entirely accurate test.

The acceptance of the Babcock method in America as a standard means of analysis led to its widespread use in practical lines of work, and it was considered reliable even for determining the fat content of buttermilk. Hunziker (5), in his first edition of "The Butter Industry," states that, "in a properly operated creamery, where the conditions relative to exhaustiveness of churning are carefully watched, the buttermilk seldom exceeds 0.2 per cent. . . ." Guthrie (6) makes no statement relative

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to the average fat content of buttermilk, but recommends the Babcock test as a means of determining the amount of fat lost in this product.

By 1921, however, gravimetric analyses of buttermilk in the laboratory of the American Association of Creamery Butter-makers had indicated that the fat content was considerably higher than can be estimated by the Babcock method. On the assumption that the gravimetric method yields correct results a modified Babcock method was devised at the American Association laboratory (7) which was named the American Association test, later known as the butyl alcohol test. This method gave results agreeing quite closely with those obtained by the gravimetric procedure. In 1925 the American Association test was accepted by the Dairy Division, University of Minnesota as the best one for use in creameries in testing their buttermilk. This acceptance was based on the same assumption that the American Association chemist made, and came as a result of numerous favorable comparisons between the American Association and gravimetric methods. In his later edition Hunziker (8) also recommends either a modification of the Babcock or an ether extraction method. McKay and Larsen (9) favor the American Association test.

#### LECITHIN AS A SOURCE OF ERROR

In the spring of 1927, while studying a different problem, one of us noticed that when powdered buttermilk is thoroughly extracted with alcohol large quantities of lecithin are removed from the buttermilk. A continuation of this work indicated that buttermilk contains much more lecithin than any of the other milk products. On reviewing the literature pertaining to the lecithin content of milk products we found that this fact had been observed at an early date but had not been considered in the more recent literature.

Dornic and Daire (10) state that buttermilk contains a higher percentage of lecithin than does any other milk product with the possible exception of cream. In this regard they cite and corroborate the work of Bordas and Racekowski (11). Dornic



and Daire (10) offer an explanation for the concentration of lecithin in buttermilk by pointing out that it is very probable that lecithin made up part of the famous "Slimmen-membran" of Storch; and that during churning, which brings the union of the fat globules, this "Slimmen-membran" disengages itself and is found later in the buttermilk. This explanation is supported by the work of Palmer and Samuelson (13) who washed fat globules and their so-called membranes free of serum by repeated dilution with water and reseparation. The buttermilk resulting from the churning of such cream was found to contain a mixture of phosphatides. It is likely that lecithin constituted a large portion of these phosphatides.

The facts and theories just pointed out suggest a reason for the disagreement between results of various methods of estimating fat content of buttermilk when applied to single samples. Lecithin as well as other lipins is soluble in ether. Consequently ether extraction as specified in the gravimetric method of estimating the fat content of dairy products may include not only fat but also lecithin in the extract. Obviously if this is the case then the gravimetric method yields results which are too high by the amount of lecithin, or other phospholipins present. The Babcock method, which always yields lower results for buttermilk than the gravimetric, may not include lecithin in the fat column. Storch (12) states that part of the "Slimmen-membran" is included in the extract obtained by the Roese-Gottlieb method causing this method to yield higher results than the Soxhlet. Lecithin may have been responsible for this observation.

If the error just discussed occurs it may be expected to be more evident in connection with buttermilk than with other products because of (1) its relatively high lecithin content and (2) its low fat content.

#### STEROLS AS A SOURCE OF ERROR

If the explanation for the concentration of lecithin in buttermilk lies in its disengagement from the surface of the fat globules in churning then another possible error in the gravimetric method can be suggested. It has been reported by Fox and Gardner

(14) that the sterol content of milk is roughly in proportion to the fat content. The probability that the sterols are concentrated at the surface of the fat globules is strengthened by the report of the above authors that the ratio of cholesterol to fat is greater in milk than in butter. Further evidence is supplied by Wacker and Beck (15) who show that in skimmilk the greater part of the cholesterol follows the cream fraction. This suggests that the cholesterol, and possibly other sterols, must be present in buttermilk in greater percentages than in milk, for the sterols may then be expected to disengage from the fat during churning in the same manner suggested for lecithin.

The sterols bear no close chemical relationship to fat aside from the fact that they may be dissolved by the same solvents. It is to be expected, therefore, that the gravimetric method in any of its variations which call for the extraction of fat from milk with ether will include the sterols with the fat in the resulting extract. On this basis the gravimetric method may be expected to yield results which are too high by the amount of the sterols present in the sample analyzed.

#### EXPERIMENTAL

*Errors in the gravimetric method due to lecithin.* In approaching this problem an attempt was made to determine the amount of lecithin occurring in the alcohol-ether-petroleum ether extract of buttermilk extracted according to the Roesse-Gottlieb directions. Macleans's (16) statement that no satisfactory method is available for the quantitative determination of lecithin in the presence of fat was substantiated by us. It was found impossible to precipitate lecithin quantitatively from its ether solutions in the presence of fat, although a copious precipitate resulting from the addition of acetone to such solutions of the extract from buttermilks demonstrated that considerable quantities of lecithin were always present. The various methods of determining the organic phosphorus of such extracts and calculating the quantity of lecithin from it are open to at least two criticisms. First, as pointed out by Maclean (16), a certain amount of inorganic phosphorus is usually included in the extract due to its solubility in the

solvents used. Second, as suggested by Grimmer (17), we have no knowledge of what fatty acids are present in the lecithin mole-

TABLE 1

*Results of various methods of fat estimation applied to synthetic milks to which fat alone was added*

MILK NUMBER	SAMPLE NUMBER	FAT ADDED	"FAT" ESTIMATION BY			
			Gravimetric method	Butyl alcohol method	Babcock method (skimmilk bottle)	Babcock method (whole milk bottle)
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
A	1	0.0000	0.0000	0.00	0.00	0.00
A	2	0.1700	0.1583	0.04	0.05	
B	3	0.0000	0.0000	0.00	0.00	0.00
B	4	0.4300	0.3864	0.22	0.22	0.43
C	5	0.1497	0.1426	0.05	0.10	0.23
C	6	0.3526	0.3508	0.29	0.24	0.40
C	7	0.5964	0.5979	0.60	0.32	0.60
D	8		0.1579	0.13	0.10	0.32
D	9		0.3017	0.30	0.24	0.40

TABLE 2

*Results of various methods of fat estimation applied to synthetic milks to which lecithin alone was added*

MILK NUMBER	SAMPLE NUMBER	LECITHIN ADDED	"FAT" ESTIMATION BY			
			Gravimetric method	Butyl alcohol method	Babcock method (skimmilk bottle)	Babcock method (whole milk bottle)
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
C	10	0.1981	0.2095	0.11	0.00	0.00
C	11	0.4000*	0.3153	0.29	0.00	0.00
D	12		0.1698	0.21	0.01	0.00
D	13		0.3006	0.44	0.04	0.00

\* In shaking this sample to dissolve the lecithin the flask was broken and a part of its contents lost. This undoubtedly accounts for the failure of the gravimetric results to agree with the calculated amount present.

cule occurring in milk, and until this is determined we are not justified in using any factor for converting percentage phosphorus to lecithin.

*Behavior of lecithin in testing.* Because of the lack of an accurate means of determining the percentage of lecithin contained in buttermilk, it was necessary to approach the problem from a different angle. Inasmuch as one of us had isolated lecithin from buttermilk, and purified it according to the directions of Maclean (16), it was proposed that by mixing known quantities of this material in a milk containing either no fat, or a known quantity of fat, it would be possible to observe its behavior when subjected to the various, common methods of determining the percentage fat content of milk. For this purpose a synthetic

TABLE 3

*Results of various methods of fat estimation applied to synthetic milks to which both fat and lecithin were added*

MILK NUMBER	SAMPLE NUMBER	FAT ADDED	LECITHIN ADDED	SUM OF FAT AND LECITHIN ADDED	"FAT" ESTIMATION BY			
					Gravi- metric method	Butyl alcohol method	Babcock method (skimmilk bottle)	Babcock method (whole milk bottle)
		per cent	per cent	per cent	per cent	per cent	per cent	per cent
B	14	0.4300	0.4700	0.9000	0.8384	0.70	0.33	
C	15	0.1497	0.2000	0.3497	0.2887	0.26	0.10	0.30
C	16	0.3524	0.4000	0.7524	0.7595	0.73	0.32	0.32
C	17	0.5964	0.3000	0.8964	0.8263	0.94	0.51	0.63
E	18	0.2467*	0.2286*	0.4753*	0.4753	0.41	0.22	0.25

\* These results calculated from the known fat content of 0.2467 per cent and the gravimetric test of 0.4753.

milk was prepared according to the directions of Clark (18). To portions of this milk additions were made of known quantities of fat, to others known quantities of lecithin, and to others known quantities of both fat and lecithin. As a check the synthetic milk without addition of fat or lecithin was also tested. The results of this work was recorded in tables 1, 2 and 3.

The methods employed for testing these products included the gravimetric, American Association or butyl alcohol and the Babcock. For the latter method results were obtained for both the whole milk and skimmed milk test bottles. All analyses were made in duplicate.

Table 1 shows the results of check tests on the synthetic milks made previous to the addition of either fat or lecithin, and also, results of tests on the same milks to which known quantities of fat had been added.

In table 2 are presented the results of the various methods of fat determination applied to synthetic milks to which known quantities of lecithin had been added.

In table 3 are presented results of the various methods of fat percentage determination applied to synthetic milks to which known quantities of both fat and lecithin had been added. These milks are thought by the authors to imitate very closely the conditions actually existing in buttermilk.

The technique of adding these substances to the synthetic milk was faulty in the cases of milks "A" and "B" as is evidenced by the disagreement between the amounts calculated to be present and the percentage found by the gravimetric method. For this reason it is the opinion of the authors that the gravimetric method represents more nearly the true fat and true lecithin content of these samples than do the calculated percentages. In the case of milk "C," however, a more accurate technique was employed, by weighing these substances on a small tin container and adding container and all to the milk, and it is seen that the gravimetric and calculated percentages agree satisfactorily. The results with milk "C" showed that the gravimetric method is accurate for determining fat or lecithin alone and consequently the gravimetric percentage is used for milks "D" and "E" as a measure of the amounts of these substances present. This was particularly necessary for milk "E" because the emulsification of the fat in this sample was accomplished by means of homogenization, whereas with the other samples it was done by shaking.

*Lecithin content of buttermilk.* As previously stated we were unable to determine accurately the percentage of lecithin present in our buttermilk because of inability to find an accurate and reliable method. Although the work with synthetic milk did not evolve any such method it did furnish us with a means of roughly measuring the lecithin content of buttermilk. It has been shown that the Babcock method estimates fat but not lecithin.

thin, whereas the gravimetric method shows both. It is therefore logical to expect that the difference between the gravimetric and Babcock analyses of a single sample of buttermilk should show the approximate percentage of lecithin present. A glance at the figures in table 4, which are calculated from the results presented in table 3, show that this calculation does give the approximate percentage of lecithin contained in these samples of synthetic milk. On this basis the percentage of lecithin in buttermilk was studied.

For this phase of the work we prefer to substitute the word "lipin" for "lecithin," because it is possible that not all of the phosphatides of milk or buttermilk are lecithin as Koch and Woods (19) state that milk contains two phospholipins, lecithin

TABLE 4

*Difference between gravimetric and Babcock results as a measure of lecithin content*

MILK NUMBER	SAMPLE NUMBER	LECITHIN ADDED	LECITHIN CALCULATED, GRAVIMETRIC MINUS BABCOCK
		<i>per cent</i>	<i>per cent</i>
B	14	0.47	0.5084
C	15	0.20	0.1887
C	16	0.40	0.4395
C	17	0.30	0.3163

and cephalin in approximately equal quantities. The word "lipin" is here used with the meaning applied to it by Maclean (16).

If we accept the suggestion of Dornic and Daire (10) that the lecithin is concentrated at the surface of the fat globules then one should expect that buttermilk from cream high in fat would contain a higher percentage of lipins than buttermilk from a cream low in fat. We therefore obtained a cream containing 52 per cent fat and churned part of it. The other part was diluted with skimmed milk to test 26 per cent and churned. The results of estimating the lipin content of buttermilks from these creams by the differences between the gravimetric and Babcock tests showed 0.59 per cent for the first and 0.36 for the second.

To confirm this result another trial was conducted in which the following buttermilks were tested:

1. From 22 per cent cream prepared by diluting 45 per cent cream with skimmed milk.
2. From 45 per cent cream.
3. From 34 per cent cream prepared by diluting 68 per cent cream with buttermilk no. 1.
4. From 68 per cent cream.

The results of this trial are recorded in table 5.

The difference between the gravimetric and Babcock results, which we believe is accounted for by the presence of lipins, is

TABLE 5

*Lipin content of buttermilks from creams of different fat content as measured by difference between gravimetric and Babcock results*

BUTTERMILK NUMBER	GRAVIMETRIC ANALYSES	BABCOCK ANALYSES	CALCULATED LIPINS
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	0.5131	0.12	0.3931
2	1.2742	0.70*	0.5742
3	9.6652	9.00*	0.6652
4	2.6768	1.80*	0.8768

\* Determined in whole milk test bottle, read with glymol.

seen to increase with the increase in the fat content of the cream from which the buttermilks were churned. We believe this indicates that our method of determining the approximate lipin content of buttermilk is roughly correct. Assuming it to be correct we find the lipin content of buttermilk to be between 0.36 and 0.87 per cent. So high a percentage of lipins in buttermilk as indicated here is sufficient to cause the gravimetric method to yield results for average buttermilk which are fully double the percentage of fat present.

*Errors in the gravimetric method due to sterols.* As we have already pointed out it is possible that an error in the gravimetric results may occur due to sterols. Sterols differ sufficiently from butterfat that if a mixture of fat and sterols are subjected to

saponification under proper conditions the fat, and likewise the lecithin if present, may be transformed into soap while the sterols are not changed. The determination of the non-saponifiable matter therefore offers a means of detecting not only the amount of sterols, but all the non-saponifiable materials extracted from milk by the solvents commonly used in the gravimetric determinations of fat percentage.

Not enough results have been secured by us to conclusively settle this question. We offer our determinations only as evidence that this error exists, and with the hope that other investigators may attempt to verify the results.

For this work it was necessary to have relatively large quantities of ether extract of buttermilk on which to make determina-

TABLE 6  
*Error in gravimetric method due to non-saponifiable matter in ether extract*

BUTTERMILK SAMPLE NUMBER	"FAT" BY GRAVIMETRIC METHOD	NON-SAPONIFIABLE MATTER IN BUTTERMILK	NON-SAPONIFIABLE MATTER IN ETHER EXTRACT
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	1.3130	0.0236	1.838
2	1.2742	0.0195	1.530
3	0.6480	0.0107	1.665
4	0.6815	0.0284	4.180

tions. Consequently, 100-gram quantities of buttermilk were extracted with alcohol, ether and petroleum ether in separatory funnels of a liter capacity. This process was repeated with each sample of buttermilk until 5 grams of extract were obtained. The official Roese-Gottlieb directions call for the use of 1.5 cc. of ammonia, 10 cc. of ethyl alcohol, 25 cc. of ethyl ether and 25 cc. of petroleum ether for 10 grams of buttermilk. Accordingly 10 times these amounts were used for this extraction. It was deemed unnecessary to reextract as required in the official directions because the yield on the second extraction was too small to warrant the use of such large quantities of solvents. It was assumed that the single extraction would yield materials which were representative of the entire amount of alcohol-ether soluble material present in buttermilk.



The percentage non-saponifiable matter contained in these extracts was determined according to the method described by the Association of Official Agricultural Chemists. The results of these studies are presented in table 6. A qualitative study of this non-saponifiable matter indicated that it was made up almost entirely of cholesterol. The cholesterol content of milk has been reported by Dennis and Minot (20) to be between 10.5 and 17.6 mgm. per 100 cc., while Fox and Gardner (14) report the amount to be 11.4 to 17.3 mgm. per 100 cc. The amount of non-saponifiable matter in butterfat centrifuged or churned from whole milk is usually very low. There can be little doubt that buttermilk contains non-saponifiable materials, chiefly cholesterol, which are responsible for an error of about 0.02 per cent in any gravimetric method which requires the extraction of the product with ether, alcohol, and petroleum ether.

#### DISCUSSION

*The gravimetric method.* Our results show that the gravimetric method of fat determination, when applied to buttermilk, gives results which are higher than the true fat content by the amount of non-saponifiable matter and lecithin present in the product tested. Tables 2 and 3 show this definitely for it is seen from table 2 that the method is as accurate for determining the percentage of lecithin as it is for determining the percentage of fat. Results recorded in table 3 further show that, rather than determining the percentage of fat in samples containing both fat and lecithin, it estimates the total of the percentages of fat and lecithin, within reasonable limits of experimental error.

*The American Association method.* Inasmuch as the American Association or butyl alcohol test determines the percentages of lecithin in synthetic milk, as shown by table 2, and determines roughly the total of the percentages of fat and lecithin when both are present it is inaccurate. The only reason for the use of this method in the past has been the close agreement of its results with those obtained by the gravimetric method. This was sufficient reason for its use, assuming the gravimetric or official method to yield correct results. On this assumption one of us

has repeatedly recommended the American Association method during the past few years for testing buttermilk. The results of the work reported in this paper, however, are considered by us to show beyond question that the use of this method should be discontinued.

*The Babcock method.* The Babcock method of fat percentage determination is shown by our results to be most accurate for the testing of a product such as buttermilk, containing roughly as much lecithin as fat. It is free from the criticism that we make of the gravimetric and American Association methods. It does fail to give entirely accurate results, however, particularly in the testing of buttermilks below 0.3 per cent fat, in which cases its results are often considerably too low. To use the whole milk test bottle yields results which are usually too high due to the inclusion of the meniscus in the reading. It will be noticed that an average of the skimmed and whole milk bottle results agrees fairly well with the actual fat content in most cases, but we do not recommend this as an accurate means of determination of the fat content of buttermilk because it averages two opposite errors.

The failure of the Babcock method to estimate all the fat in the cases cited above recalls the work of Farrington (21) who was the first to report that by using more sulphuric acid and centrifuging for a full five minutes at the prescribed speed higher results may be obtained. Our studies with synthetic milk explain why this happens for if we subject a test of synthetic milk, to which lecithin but no fat has been added, to these conditions we obtain a dark but definite column in the neck of the bottle which is undoubtedly either lecithin or its decomposition products. At one time we subjected some Babcock tests of buttermilk to extremely high centrifugal force for prolonged periods and obtained results which agreed closely with the gravimetric results. It is evident, therefore, that the so-called "rigorous method" of Farrington may include some lecithin in the fat column along with the fat. This possibility is strengthened by the evidence supplied by Coriat (22) that lecithin is not easily hydrolized by acids. If this is true then a part of the lecithin exists in the Babcock test acid mixture without change.

*The Gerber method.* It is well known that in the European countries the Babcock method has never been accepted as a standard "quick" test. Instead, these countries have adopted the Gerber method which makes use of amyl alcohol in much the same manner that the American Association test uses butyl alcohol. Amyl alcohol differs from butyl in that it is the next higher member of the series. Its properties are little different from those of butyl alcohol and its effect when used in a milk test such as the Gerber is essentially the same as that of butyl alcohol. The authors are therefore of the opinion that the Gerber method also yields incorrect results for buttermilk.

#### REFERENCES

- (1) BABCOCK, S. M.: A new method for the estimation of fat in milk especially adapted to creameries and cheese factories. Wis. Exp. Sta. Bull. 24, 1890.
- (2) MYERS, J. A.: Methods of testing milk. 3rd annual report, W. Va. Exp. Sta., 1891, pp. 68-87.
- (3) RÖMSE, B.: Zur Analyse der Milch. Fettbestimmung., Z. Angewand Chem., 1888, i, 100.
- (4) GOTTLIEB, E.: Eine bequeme Methode zur Bestimmung von Fett in Milch. Landw. Vers.-Stat., 1892, xl, 1.
- (5) HUNZIKER, O. F.: The Butter Industry, ed. 1, 1920. O. F. Hunziker, La Grange, Ill., p. 402.
- (6) GUTHRIE, E. S.: The Book of Butter, Revised, 1923. The Macmillan Company, New York, p. 251.
- (7) MITCHELL, J. W.: Churning losses. Chicago Dairy Produce, December 13, 1921, p. 16.
- (8) HUNZIKER, O. F.: The Butter Industry, ed. 3, 1927. O. F. Hunziker, La Grange, Ill., p. 307.
- (9) MCKAY, G. L., AND LARSEN, C.: Principles and Practices of Buttermaking, ed. 3, 1922. Wiley and Sons, New York, p. 101.
- (10) DORNIC, M. M., AND DAIRE: La Contribution à la Étude de la Lecithin du Lait. Ann. des Falsif., 1910, iii, 533.
- (11) BORDAS, F., AND DE RACYKOWSKI, SIG.: Sur le Dosage de la Lecithin dans le Lait. Compt. Rend. Acad. Sci., 1902, cxxxiv, 1592.
- (12) STORCH, V.: Untersolgelser over: Konsistensfejl hos Smorret og Aarsagerne til deres Fremkomst, samt over: Mælkekuglernes Rygning, Den kgl. Veterinar- og handbohojskoles Laboratorium for Landokono-miske Forsog 1897, 36th Beret.
- (13) PAEMER, L. S., AND SAMUELSON, E.: The nature of the substances adsorbed on the surface of the fat globules in cow's milk. Proc. Soc. Exp. Biol. Med., 1924, xxi, 537.

- (14) FOX, F. W., AND GARDNER, A. J.: The sterol content of cow's milk. *Biochem. Jour.*, 1923, xvii, 288.
- (15) WACKER, L., AND BECK, K. F.: Untersuchen über den cholesteringehalt von Frauen- und Kuhmilch. *Z. Kinderheilk*, 1921, xxvi, 288.
- (16) MACLEAN, H.: *Lecithin and Allied Substances: The Lipins*. Longmans, Green and Company, New York, 1918.
- (17) GRIMMER, W.: *Milchwirtschaftliches Practicum*. Akademische. Verlagsgesellschaft, Leipzig, 1926, p. 75.
- (18) CLARK, W. M.: "Synthetic milk" as a basis for research. *Jour. Dairy Sci.*, 1927, x, 195.
- (19) KOCH, W., AND WOODS, H. S.: The quantitative estimation of the lecithans. *Jour. Biol. Chem.*, 1906, i, 205.
- (20) DENNIS, W., AND MINOT, A. S.: Cholesterol in milk. *Jour. Biol. Chem.*, 1918, xxxvi, 58.
- (21) FARRINGTON, E. H.: A comparison of the Babcock test and the gravimetric method of estimating fat in skim milk. *Wis. Exp. Sta. Bull.* 52, 1896.
- (22) CORIAT, J. H.: Production of cholin from lecithin and brain tissue. *Amer. Jour. Physiol.*, 1904, xii, 353.

# A COMPARISON OF CERTAIN METHODS FOR DETERMINING THE SANITARY QUALITY OF ICE CREAM\*

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## INTRODUCTION

From a sanitary point of view the production of all dairy foods requires the solution of two problems, viz.: (a) to avoid the danger of conveying disease, and (b) to avoid unsanitary conditions. Sanitarians have devoted especial attention to the production of safe and clean milk, but they have not given the same consideration to the manufacture of ice cream. Not only does the use of ice cream involve the same dangers as the use of milk but, in addition, those that may be introduced while dispensing it. Frequently ice cream dippers, dishes and hands are washed in the same water, which is used without heat or sterilizing agents and changed at infrequent intervals. Nor is the problem of eliminating unsanitary conditions less important than in milk, even if undesirable flavors can be successfully hidden by sugar, essences, fruits or nuts. Responsibility for these conditions seems to rest with the scientists who have failed to provide the necessary technic for determining unsanitary conditions; with the sanitarians who have been less exacting in their requirements; and with the legislators who have placed undue emphasis on the butter fat content and too little on the manner of handling of the product.

There are three bacteriological tests available for determining the sanitary quality of ice cream: (a) total count of bacteria; (b) colon group test; and (c) anaerobic spore test. At present the total count of bacteria is used to the exclusion of the other two, and commonly not even this test is required. It would seem

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that ice cream merits greater consideration by sanitarians than it has received, and that a comparative study of the methods available for determining its sanitary quality is highly desirable.

#### HISTORICAL

In the past investigators seem to have centered their efforts chiefly upon the bacterial count of ice cream. As early as 1904, Buchan (1) suggested that ice cream should not contain more than 1,000,000 organisms per cubic centimeter. Fay (2) believed that ice cream could be produced in Kansas with a bacterial count of 100,000 or less if pasteurization and care in handling were practiced. The next year Fay and Olson (3) went a step farther and declared it practical to produce ice cream containing less than 100,000 bacteria per gram. In Michigan, Fabian made a number of helpful studies: In 1920 he (4) suggested a score card for ice cream plants; with Cromley in 1923 he (5) studied the influence of manufacturing operations on the bacterial count of ice cream; in 1926 he (6) showed the relation of ice cream to disease epidemics; and the same year he (7) suggested a bacteriological standard of 100,000 or less per gram. Apparently only one state, California (8), has placed a legal standard upon the bacterial count of ice cream, namely, a maximum count of 150,000 per gram. The colon group determination has not been employed for ice cream, and the anaerobic spore test devised for milk has never been attempted.

The opinion voiced editorially (9) in 1920 in the *Journal of the American Medical Association* still seems applicable: "Is it unfair to say that better standards should be maintained in this industry which has heretofore often escaped suspicion because of a mistaken belief in the bactericidal effect of cold?"

#### METHODS EMPLOYED

The samples were placed in an incubator at 37°C. for about twenty minutes to melt the cream so that it could be measured with graduated pipettes for making the necessary dilutions and cultures. All samples were analyzed within an hour after they were dispensed.

*For the total count a dilution of 1:10,000 was used. Plating a larger number of dilutions would have given more accurate results. Duplicate plates were made, incubated forty-eight hours at 37°C., and the colonies counted by means of a hand lens.*

Since those who have made the most intensive studies of ice cream agree that a standard of 100,000 or less could readily be maintained, and since California has actually adopted 150,000 as a permissible maximum, we felt that 500,000 would furnish a very liberal basis for condemnation purposes. Accordingly this figure was arbitrarily applied to the results.

*For the colon group determination the dilutions employed were 1:10, 1:100, to 1:1,000,000. Enrichment tubes containing 1 per cent lactose bouillon were inoculated with 1 cc. portions from each dilution. After enrichment, endo agar plates were made from tubes showing 10 per cent or more gas. Typical colonies were fished and confirmed according to the American Public Health Association Standard Methods for Water Analysis.*

There being no set standard for the permissible number of colon group bacteria allowable in ice cream, and none suggested so far as we were aware, we finally decided that a density of 10,000 per cubic centimeters would afford a liberal limit which was arbitrarily adopted.

*For the anaerobic spore test, 1 cc. of the ice cream was placed in each of five sterile tubes containing vaseline and about 10 cc. of sterile water added. These tubes were heated at 80°C. for ten minutes to kill vegetative bacteria, expel oxygen, and bring the vaseline to the surface thus making the anaerobic seal. All cultures were incubated at 37°C. for 96 hours. This was essentially the technic described by Weinzirl (10) for milk work, the 1 cc. of ice cream being substituted for 5 cc. of milk. For purposes of condemnation, an arbitrary standard based upon our limited experience, was set up. It appeared probable that three positives out of five 1 cc. portions would catch excessive pollution and eliminate anaerobes added with the sugar.*

## RESULTS OBTAINED

By the methods outlined above 124 samples of ice cream, including all the common varieties, were tested. Of these samples 24 came directly from the factory, and 100 from dispensers. The data are too bulky to be included in full, but summaries will be given for the separate tests.

The factory samples gave very low counts, 100 per cent of them being less than 500,000. Of the dispensers' samples only 31 per cent fell below 500,000, while 69 per cent were above. (See table 1.) Since most of the ice cream dispensed was produced in the factories considered, and since 94 per cent of the samples were pasteurized, it appears obvious that there is a tremendous

TABLE 1  
*Summary of results when ice cream was tested by the total count method*

SOURCE OF SAMPLES	NUMBER OF SAMPLES	COUNT LESS THAN 100,000	COUNT MORE THAN				
			100,000	500,000	1,000,000	10,000,- 000	100,000,- 000
Factory. ....	24	21	3	0	0	0	0
Dispenser. ....	100	4	96	69	37	8	4
Total.....	124	25	99	69	37	8	4

increase after the product left the factories due either to multiplication or contamination or both.

This difference between factory and shop samples was a great surprise to us, but according to the literature, it appears that similar results were obtained by other investigators. It seems quite clear that the factories can readily meet a standard of 500,000, especially if they are permitted to pasteurize their product. For the dispensers to meet this standard it would require radical improvement in their methods.

Assuming that the presence of colon group in a dilution of 1:10 means a density of 10 per cubic centimeter, etc., and recalling that the arbitrary standard adopted was a density of 10,000 per cubic centimeter, we find that 92 per cent of the factory



samples meet this requirement, while only 68 per cent of the dispensers' samples meet it. (See table 2.) This shows a disparity between the two sources similar to that shown by the total count, but the difference is less marked, probably justly so. The data also seem to indicate that the colon group test might prove valuable for checking the sanitary methods of the dispensers.

The anaerobic spore test was devised for the purpose of determining contamination in milk. (See table 3.) Obviously it

TABLE 2

*Summary of results when ice cream was tested by the colon group determination*

SOURCE OF SAMPLES	NUMBER OF SAMPLES	COLON GROUP PRESENT IN DILUTION					
		1:10	1:100	1:1000	1:10,000	1:100,000	1:1,000,000
Factory.....	24	21	17	11	2	0	0
Dispenser.....	100	89	77	58	32	16	9
Total.....	124	110	94	69	34	16	9

TABLE 3

*Summary of results when ice cream was tested by the anaerobic spore test*

SOURCE OF SAMPLES	NUMBER OF SAMPLES	NUMBER OF TUBES SHOWING ANAEROBIC SPORES				
		1	2	3	4	5
Factory.....	24	6	5	1	1	1
Dispenser.....	100	75	23	14	6	5
Total.....	124	81	28	15	7	6

can be applied to ice cream for the same purpose. The tests recorded above give the results which in a general way parallel those of the other two methods. Again the factory samples make the better showing, but the contrast is much less marked. It is improbable that the number of anaerobic spores increases in the frozen ice cream; if this be true, then contamination during dispensing must account for the difference. Perhaps the lower percentage of condemnation, 4 and 14 per cent, is due to

the fact that this test is probably not influenced by multiplication as are total count and colon group. If so, this seems to be a distinct advantage.

On the basis of the arbitrary standards previously suggested, we find that the total count condemns more samples than either of the other two methods, and that the colon group determination condemns more than the anaerobic spore test. (See table 4.) Obviously the standards for condemnation could have been adjusted so as to reverse these figures. It is quite impossible to determine on the basis of figures like the above which method is the more accurate for determining contamination. To decide this question it would be necessary to contaminate sterile prod-

TABLE 4  
*Summary of results using arbitrary standards for condemnation*

SOURCE OF SAMPLES	NUMBER OF SAMPLES	NUMBER OF SAMPLES CONDEMNED BY		
		Total count (500,000)	Colon group in 1:10,000	Anaerobic spores in 3 out 5, 1 cc. portions
Factory .....	24	0	2	1
Dispenser .....	100	69	32	14
Total.....	124	69	34	15

ucts and then apply the tests to see which gives the more accurate results. In the absence of such data we are compelled to base judgment of their relative values upon general considerations.

For determining contamination the total count and colon group determination cannot be accurate because both are affected by growth and multiplication of the bacteria present. It would seem unjust to condemn a given lot of ice cream as unduly contaminated when the high test is due largely to multiplication. These tests are inapplicable for a pasteurized product since pasteurization destroys the vegetative organisms. On the basis of these two tests, the pasteurized samples would have to be

regarded as exceptionally clean, while the unpasteurized would be highly unclean, although the reverse may have been true.

If the total count and the colon group test have any value in the case of pasteurized ice cream, this value is limited to determining the efficiency of pasteurization or the extent of subsequent contamination. If the high results obtained for the dispensers' samples are due partly to methods of handling, 94 per cent of them being pasteurized, then these two tests are worth while for this purpose at least. The anaerobic spore test also gives higher results for the dispensers' samples, 14 per cent as against 4 per cent.

As applied to ice cream the anaerobic spore test has the disadvantage of including the spore anaerobes present in sugar and possibly other products (10). One of us, Weinzirl (11), has shown that sugar commonly contains anaerobic spores to the extent of one per gram of sugar. If the ice cream contains 15 per cent of sugar, on the basis of this test there is introduced roughly an apparent error of 15 per cent. Actually the error cannot be so large under the standard assumed because the rule of "3 positives out of 5" tubes would largely eliminate this error. However, it is an error that cannot be disregarded.

#### SUMMARY AND CONCLUSIONS

1. A total of 124 samples of commercial ice cream were obtained from producers and retailers in Seattle and subjected to the following tests: (a) total count of bacteria; (b) colon group determination; and (c) anaerobic spore test.

2. The total count reveals the results of contamination and of subsequent multiplication of the bacteria introduced, but it does not distinguish between the two.

3. The colon group test functions like the total count, but it is somewhat more specific in indicating unsanitary conditions.

4. The anaerobic spore test shows unsanitary conditions only, but in the case of ice cream it fails to distinguish between the spores introduced through unsanitary conditions and those added with the sugar.

5. For freshly pasteurized products the first two methods are

useless except for controlling pasteurizing efficiency; they have distinct value for testing subsequent contamination; the last method reveals contamination in both pasteurized and unpasteurized products.

## REFERENCES

- (1) BUCHAN, G. F.: The contamination of ice cream. *Jour. Hyg.*, 1904, x, 93-130.
- (2) FAY, A. C.: The bacterial content of some Kansas ice cream. *Jour. Dairy Sci.*, 1923, vi, 283-291.
- (3) FAY, A. C., AND OLSON, N. E.: The bacterial content of ice cream. *Jour. Dairy Sci.*, 1924, vii, 330-356.
- (4) FABIAN, F. W.: A score card for city ice cream plants. *Jour. Dairy Sci.*, 1920, iii, 230-235.
- (5) FABIAN, F. W., AND CROMLEY, R. H.: The influence of manufacturing operations on the bacterial content of ice cream. 1923. *Tech. Bul. 60*, Mich. Agr. Exp. Sta.
- (6) FABIAN, F. W.: Ice cream as a cause of epidemics. *Jour. Amer. Pub. Health Assoc.*, 1926, xvi, 873-879.
- (7) FABIAN, F. W.: A suggested bacteriological standard for ice cream. 1926. *Sp. Bul. 158*, Mich. Agr. Exp. Sta.
- (8) FREY, J. J.: California's new bacterial standard (for ice cream). *Western Confectioner*, 1927, xiv, 36.
- (9) Editorial. Ice cream and bacteria. *Jour. Amer. Med. Assoc.*, 1917, lxviii, 1263.
- (10) WEINZIRL, JOHN: Detection of manural pollution in milk by the anaerobic spore test. *Amer. Jour. Pub. Health*, 1921, xi, 149-153.
- (11) WEINZIRL, JOHN: Sugar as a source of the anaerobes causing explosion of chocolate candies. *Jour. Bact.*, 1927, xiii, 203-207.

# THE EFFECT OF FLASH PASTEURIZATION OF MILK UPON THE FLAVOR AND TEXTURE OF CHEDDAR CHEESE\*

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A considerable amount of cheddar cheese of poor quality is manufactured in the Western states, especially during the summer months, since proper care is not given to the milk by the producers; consequently, the supply is not uniform throughout the year, and usually varies during a season. Cheese is the only dairy product which can be manufactured in California from milk produced from herds infected with tuberculosis without pasteurization of the milk.

Due to these conditions experiments were started at the California Experiment Station at Davis in October, 1925, in order to determine the advisability of flash pasteurization at different temperatures in the cheddar cheese process. The process is not new, since there are several references in the literature. Sammis and Bruhn (1) in 1912 reported the successful manufacture of cheddar cheese from milk flash pasteurized at 160° to 165°F. for an instant. They added hydrochloric acid to the milk in order to aid the coagulation by rennet. This method evidently has not been used to any extent in commercial manufacture during recent years.

Stevenson in 1920 (2) and in 1923 (3) discussed the methods used in New Zealand, where approximately two-thirds of all cheddar cheese manufactured is from milk pasteurized by the flash method. Regenerative flash pasteurization was used, heating the milk to 160° to 165°F., and cooling to the setting temperature. Temperatures below 160°F. allowed a deterioration in the flavor of the cheese, and if above 165°F., the body and tex-

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ture of the cheese were injured, the result being mealy cheese. Hydrochloric acid was found to be of no assistance in aiding coagulation. The cheese made from pasteurized milk scored two to three points higher after this practice was started.

Murray (4) in 1924, states that in experiments at the Hawkesbury Agricultural College and Moruya Cooperative Cheese factory it has been found that flash pasteurization at 155°, 165° and 175°F., or holding at 145°F. for 30 minutes tends to control the fermentation of the milk to be used in cheese making, and also reduces the undesirable organisms present. The quality of the cheese was thereby improved.

Considerable experimental work using the holding method of pasteurization has been reported. Price (5) in 1927 gave a summary of this work, and also reported the results of rather extensive work at the New York Agricultural Experiment Station and recommended this method.

The flash method of pasteurization seems to be well suited to the factories operating in the Western states, and consequently the holding method was not included in the scope of this work.

## EXPERIMENTAL

### *Use of good milk*

Raw milk of good quality was selected at the University Farm Creamery platform. The bacterial counts varied from 8500 to 113,000 per milliliter, and there were no gas-forming organisms present as shown by the Wisconsin curd test. Four trials were made; 800 pounds of milk being selected each time. The milk was run into a receiving vat and was mixed thoroughly by means of a horizontal coil which operated continuously.

A check vat of cheese was made, using 400 pounds of the raw milk according to the regular methods of procedure. The remaining 400 pounds of milk were run over a forewarmer which heated the milk to 110°F., then through a horizontal flash pasteurizer of 3500 pounds capacity per hour, where it was heated from 160° to 168°F. An automatic temperature control apparatus was attached to the pasteurizer and the temperature

was held within the above range. The milk was cooled to 88°F. by flowing over a surface cooler. The sanitary pipe line from the pasteurizer to the cooler was rather long, and the milk was held at the pasteurization temperature approximately 25 seconds.

The cheese was made in the regular manner except that one ounce more rennet was used for each 1000 pounds of milk and a setting temperature of 88°F. was used. The cheese from the four lots was cured at 50°F., and scored at intervals by members of the Dairy Industry Division of the University of California,

TABLE 1  
*Flavor and texture scores on cheese made from milk of good quality*

LOT	MILK	AGE—2 MONTHS		AGE—6 MONTHS	
		Flavor score	Texture score	Flavor score	Texture score
1	Raw	38.0	29.0	39.0	29.5
1	Pasteurized	36.5	27.5	38.5	29.0
2	Raw	38.5	29.0	36.0	29.5
2	Pasteurized	38.25	29.0	38.5	30.0
3	Raw	37.0	29.5	36.0	29.5
3	Pasteurized	36.0	29.0	37.0	29.5
4	Raw	37.5	28.0	39.0	29.0
4	Pasteurized	37.5	29.5	36.5	29.0
Average:					
Raw.....		37.75	28.87	37.50	29.37
Pasteurized.....		37.12	29.00	37.62	29.37

and by representatives of the Bureau of Dairy Industry, United States Department of Agriculture. The score card recommended by the United States Department of Agriculture was used which allows 45 points on flavor and 30 points on texture. There were no uniform criticisms on flavor and texture, and the scores are given in table 1.

Neither the scores nor the criticisms show uniform improvement or defects caused by the pasteurization. The texture was fairly uniform however, and did not seem to be affected by heating the milk.

*Use of inferior milk*

Milk of poor quality was selected from the creamery platform. The flavor and odor were bad, and large numbers of gas-forming organisms were shown to be present by the Wisconsin curd test. The acidity was low, however, ranging from 0.16 to 0.18 per cent. The average bacterial count on the seven lots of milk was 410,000 per milliliter, and after heating was 5100 per milliliter, giving an

TABLE 2  
*Flavor and texture scores on cheese made from milk of poor quality*

LOT	MILK	AGE—2 MONTHS		AGE—6 MONTHS	
		Flavor score	Texture score	Flavor score	Texture score
5	Raw	34.0	27.0	32.0	27.0
5	Pasteurized	40.0	29.0	38.5	29.0
6	Raw	34.0	29.0	33.0	29.0
6	Pasteurized	40.0	29.5	39.5	29.5
7	Raw	34.5	29.0	35.0	29.0
7	Pasteurized	39.0	29.0	38.5	29.5
8	Raw	35.0	29.5	32.0	29.5
8	Pasteurized	39.0	29.5	39.0	29.5
9	Raw	34.0	29.0	34.0	29.5
9	Pasteurized	38.0	29.5	38.5	29.5
10	Raw	37.0	29.0	34.0	29.0
10	Pasteurized	38.0	29.5	40.0	29.5
11	Raw	34.5	28.0	35.0	28.5
11	Pasteurized	38.0	29.0	36.5	29.5
Average:					
Raw. . . . .		34.71	28.64	33.59	28.78
Pasteurized . . . . .		38.86	29.28	38.64	29.43

average efficiency of 98.76 per cent at an average temperature of 165°F.

A composite portion of 100 pounds of milk was taken for the check vats. A larger portion of the milk, 3300 pounds for each lot, was heated. The flavor and texture scores are given in table 2.

Table 2 shows improvement in the flavor score of the cheese in every case when milk of poor quality was heated, the average difference in scores being approximately four points at two



months and five points at six months. A large portion of the cheese made from raw milk was not marketable due to bitter and unclean flavors. The texture was improved by heating the milk, as the formation of gas holes was eliminated.

The average yield of the cheese manufactured from the heated milk was 9.49 pounds for each 100 pounds of milk testing 3.34 per cent butterfat. Yield was not considered in the case of the raw milk because the amount of milk used was too small to insure valuable data. The average butterfat loss in the whey from the heated milk was 0.169 per cent and from the raw milk 0.222 per cent.

TABLE 3

*Flavor and texture scores on cheese made from milk pasteurized at 177°F. by the flash method*

LOT	MILK	AGE—2 MONTHS		AGE—6 MONTHS	
		Flavor score	Texture score	Flavor score	Texture score
12	Pasteurized	37	28.5	36	29.0
13	Pasteurized	36	29.0	37	29.0
14	Pasteurized	37	29.0	36	29.0
15	Pasteurized	37	28.5	38	28.5
Average: Pasteurized.....		36.75	28.75	36.75	28.87

Since a minimum flash pasteurization temperature of 176°F. is recognized in the butter industry, four lots of milk were pasteurized at a temperature of 176° to 178°F. The milk was of poor quality, containing large numbers of gas forming bacteria. Check vats were not made as the foregoing data showed that the cheese made from such milk was not marketable. Four vats of milk of 2650 pounds each were made; the results are given in table 3.

Table 3 shows that cheese of fair quality could be manufactured from poor milk pasteurized at 176° to 178°F. Scorched and bitter flavors were noticeable and the texture was weak and brittle. However, the cheese was marketable, which was not the case of the raw milk cheese manufactured from similar milk.

As a result of these experiments, since July, 1926, all milk for cheddar and Monterey cheese manufacture at the University Farm Creamery has been heated by the flash method to a temperature of 160° to 170°F. The cheese at two months has a uniform mild flavor and the trade has accepted it without any returns. After six months' curing, a clean, mild characteristic, aged cheddar flavor usually develops.

The following procedure is recommended:

1. Heat milk, flashing to 160° to 168°F.
2. Cool immediately to 88°F., the setting temperature.
3. Add from 0.5 to 1 per cent clean, active starter.
4. Add 4 to 6 ounces rennet at 0.17 to 0.20 per cent acidity.
5. Coagulation period, 25 to 35 minutes.
6. Cooking temperature 100° to 104°F.
7. Acidity at dipping, 0.145 to 0.155 per cent.

#### SUMMARY

A maximum flash temperature of 168°F. may be used in heating milk for the manufacture of cheddar cheese. Higher temperatures give a weak texture, a scorched flavor and sometimes allow a bitter flavor to develop.

Heating milk to 160° to 168°F. improves the quality of the cheese in the case of poor milk, although uniform improvement is not noticed in the case of milk of good quality. Gassy milk pasteurized to 176°F. produces cheese of better quality than if the raw milk were used.

Greater uniformity in the cheese is obtained by heating the milk. A yield of 9.49 pounds of cheese for each 100 pounds of milk testing 3.34 per cent butterfat was obtained. The loss of butterfat in the whey was 0.053 per cent lower when the milk was heated.

#### REFERENCES

- (1) SAMMIS, J. L., AND BRUHN, A. T. 1912 Manufacture of cheddar cheese from pasteurized milk. Wisconsin Agri. Experiment Station Bulletin 27.
- (2) STEVENSON, C. 1920 Pasteurization in cheese manufacture. New Zealand Jour. Agr., xx, no. 1.

- (3) STEVENSON, C. 1923 Pasteurization of milk for cheddar cheese making in New Zealand. *Proceedings of the World's Dairy Congress*, vol. 1.
- (4) MURRAY, J. K. 1923 Pasteurization of milk for cheddar cheese making; the bacteriological aspect. *Agr. Gaz.*, N.S. Wales, xxxiv, no. 8, (Abs. in *Exp. Sta. Rec.*, v, no. 7).
- (5) PRICE, WALTER V. 1927 The manufacture of cheddar cheese from milk pasteurized by the holder method. *Cornell Memoir* 105.

# EFFECT OF HOMOGENIZING ICE CREAM MIXES BEFORE AND AFTER THE ADDITION OF GELATIN OR SUGAR AND BEFORE AND AFTER CONDENSING\*

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It is generally recognized that variations sometimes occur in the properties of ice cream mixes from unknown causes. Observations made from studies at this Station and from commercial practice prompted a study of certain conditions during homogenization which gave some promise of giving information on these problems. The investigation dealt with the influence of adding gelatin or sugar to the mix prior to pasteurization and homogenization as compared with the addition of these ingredients immediately after homogenization. The study also included the effect of homogenization of the mix before and after condensing.

## EXPERIMENTAL METHODS

The correct proportioning of the ingredients in the mix was calculated by the method of Price (1). Cream containing 30 per cent of fat, condensed skim-milk, water, 0.5 per cent of a medium grade gelatin and 14 per cent of sugar were the ingredients. The percentages of fat and serum solids in the finished ice cream were 12 and 10, respectively. The cream was separated from the milk of the Station Jersey herd, and the skim-milk was condensed to contain 20 to 25 per cent total solids in large flasks at a temperature of 43°C. under 27 to 28 inches of vacuum.

The ice cream mixes were pasteurized at 62° to 64°C. (143° to 147°F.) for thirty minutes or at 65°C. (149°F.) for twenty minutes. From 50 to 150 pounds of mix were required for one experiment, while for each individual batch not less than 15 pounds were required. The mixes were poured directly into a funnel attached to a Manton-Gaulin homogenizer of 60 gallons per hour capacity.

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They were processed at the pasteurization temperature and usually at 2500 pounds pressure. The mixes were cooled in cans placed in cold water and were aged at 3° to 5°C. (37° to 41°F.) for fifteen to twenty hours prior to freezing. The mixes were frozen for uniform periods of time in one gallon experimental freezers which permitted triplicate freezings under identical conditions. The brine temperature was controlled within 0.2°C. and the temperature of the finished ice cream varied half this amount.

The viscosity of the mix was measured with the MacMichael viscometer using the number 22 and 26 wires which had been standardized by oils of known viscosity furnished by the United States Bureau of Standards. The results obtained were expressed as viscosity in centipoises even though plasticity may have influenced the readings. No better means of determining or stating the fluid characteristics of the mixes was available. The temperature of the mix when viscosity determinations were made varied from 3° to 5°C. but was uniform for each series.

The fat globules were measured at a magnification of approximately 2000 diameters using an ocular micrometer disc standardized with the microscope so adjusted that each of the smallest marks represented 0.5 micron. One-half cubic centimeter of the mix to be examined was diluted with 100 cc. of distilled water and mounted as a hanging drop preparation.

The ice cream samples were scored for body, texture, and flavor by A. C. Dahlberg and the author, without knowing their identity. Hardness tests were made after several days aging in the hardening room at -17.8° to -21.1°C. (0° to -10°F.) by the method of Perkins (2). Six tests were made on one brick and the results were calculated to grams required to displace 1 cu. mm. The melting resistance of the ice cream was determined by hourly weighing duplicate pint bricks at room temperature, as previously done by Holdaway and Reynolds (3), and others.

## EXPERIMENTAL RESULTS

*Effect of homogenizing ice cream mixes with and without gelatin*

Gelatin is now usually added to the mix in the dry form prior to homogenization, but in former years it was usually dissolved in water and added to the mix after homogenization. Downey (4) favored the addition of the dry gelatin to the mix before pasteurization, although his results show that the addition of gelatin after homogenization produced slightly harder ice cream of greater melting resistance. Ambrose (5) reported ice cream to

TABLE 1

*The effect of homogenizing the ice cream mix with and without gelatin on its viscosity*

DATE	DESCRIPTION OF VARIATION IN MIXES		VISCOSITY OF MIXES	
	Per cent gelatin	Homogenization pressure	Homogenized with gelatin	Gelatin added after homogenization
		pounds per square inch	Cp.	Cp.
January 29.....	0.3	4,500	1,220	1,570
January 29.....	0.3	4,500	2,070	3,640
February 20.....	0.3	4,500	2,260	2,800
March 4.....	0.5	2,500	1,220	1,220
March 11.....	0.5	2,500	788	716
April 16.....	0.5	2,500	694	800
May 21.....	0.5	2,500	398	426

be smoother in texture and possessed greater melting resistance when the gelatin was added after homogenization.

In this study gelatin was dissolved in water and added before pasteurization and homogenization, and before and after aging. Controls were also prepared without gelatin. In experiments dated January 29 and February 20, 0.3 per cent of high-test gelatin was used but in all other experiments 0.5 per cent of a medium-grade (about 140 grams by the Bloom method) was used. The viscosities of the aged mixes are given in table 1. Although the mixes in each series are quite uniform in viscosity, it is evident that the homogenization of gelatin in the mix results

in a lower viscosity. Gelatin added to the mix just prior to freezing gave reduced viscosities, the extent depending upon the time elapsing between the addition of gelatin to the cold mix and the making of the determination.

A sufficient number of microscopic fields were examined to permit the measurement of 100 individual fat globules and all

TABLE 2

*The effect of homogenizing the ice cream mix with and without gelatin on the size and clumping of the fat globules*

DATE	AVERAGE SIZE OF FAT GLOBULES IN MICRONS		NUMBER OF CLUMPS FOUND PER 100 INDIVIDUAL GLOBULES		AVERAGE SIZE OF CLUMPS IN MICRONS	
	Before freezing	After freezing	Before freezing	After freezing	Before freezing	After freezing
Homogenized with gelatin						
April 8.....	1.74	1.47	23	22	4.87 x 3.10	3.27 x 2.50
May 14.....	1.32	1.35	52	35	4.00 x 2.90	3.00 x 2.20
May 21.....	1.56	2.05	23	31	8.36 x 5.19	8.22 x 5.48
July 1.....	1.72	1.84	36	40	4.88 x 2.70	2.75 x 1.95
July 2*.....	1.72	2.16	36	21	4.88 x 2.70	2.50 x 1.95
Average .....	1.58	1.77	33.5	29.8	5.52 x 3.47	3.95 x 2.81
Homogenized without gelatin						
April 8.....	1.32	1.42	20	10	3.15 x 2.00	2.45 x 1.70
May 14.....	1.21	1.52	31	29	2.80 x 1.90	2.30 x 1.78
May 21.....	1.89	1.95	25	17	6.74 x 4.10	6.70 x 4.60
July 1.....	1.65	1.74	32	21	4.50 x 2.70	3.14 x 2.12
July 2*.....	1.65	1.87	32	21	4.50 x 2.70	2.73 x 1.88
Average.....	1.51	1.70	27.0	24.5	4.29 x 2.67	3.46 x 2.41

observed clusters were counted and measured. In this manner the relative number of individuals and clusters was obtained. The size of the clumps was obtained by averaging together all the largest dimensions and then all the small dimensions. The data, presented in table 2, show that the presence of gelatin during homogenization tended to increase the size of the fat clumps to a

slight extent. The size of the clusters of fat globules was reduced when the mixes were frozen, but this effect was not uniform for mixes homogenized with or without gelatin.

The addition of gelatin prior to or immediately following homogenization did not alter those properties of the mix which affect its freezing and whipping characteristics. So far as could be observed or determined the mixes handled similarly in the

TABLE 3

*The effect of the time gelatin is added to the mix on the melting resistance of the ice cream*

DATE	LOSS IN WEIGHT											
	Homogenized with gelatin			Gelatin added immediately after homogenization			Gelatin added just before freezing			No gelatin added		
	First hour	Sec-ond hour	Total	First hour	Sec-ond hour	Total	First hour	Sec-ond hour	Total	First hour	Sec-ond hour	Total
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams
January 15.....	0.0	74.5	74.5	10.2	74.3	84.5	26.15	105.7	131.8			
January 29.....	50.9	119.1	170.0	41.2	140.0	181.2	62.3	134.6	196.9			
February 20.....	41.0	122.5	163.5	32.0	141.7	173.7	60.9	137.0	197.9	82.0	124.8	206.8
March 4...	51.6	69.0	120.6	46.2	99.9	146.1	61.7	93.0	155.7	84.0	87.7	171.7
March 11	41.9	69.5	111.4	28.2	82.5	110.7	68.6	91.0	159.6	86.4	78.5	164.9
March 18	21.7	86.0	107.7	14.8	91.0	105.8	32.6	106.3	138.9	68.4	89.3	157.7
April 16..	61.7	105.7	166.8	61.0	102.1	163.1	85.2	100.5	185.7	103.4	62.4	165.8
May 12 ...	54.9	117.6	172.5	67.6	115.7	183.3	112.4	104.6	217.0			
May 21 ...	25.9	120.0	145.9	35.2	130.3	165.5						
Average	38.8	98.2	136.9	37.3	108.6	145.7	63.7	109.0	172.9	84.8	88.5	173.3

freezers and gave the same overrun under uniform conditions. It seemed unnecessary to present these data.

The ice cream made from the mix to which the gelatin was added immediately after homogenization was placed first for texture in seven out of nine trials and was never placed third or fourth. The ice cream made from the mix homogenized with gelatin was placed first once and was second and third in a total of



eight trials. The evidence indicates that the action of gelatin is slightly stronger when added immediately after homogenization, but this difference is not pronounced and may not be sufficient to be a factor to consider commercially. It is interesting to note that ice cream to which the gelatin was added just prior to freezing was usually scored third, thus demonstrating the importance of aging gelatin in the ice cream mix. The ice cream containing no gelatin was always placed fourth not only because of coarse texture, but also because the body was crumbly and unfrozen syrup often drained from it during storage.

TABLE 4

*The effect of the time gelatin is added to the mix on the hardness of the ice cream*

DATE	HARDNESS IN GRAMS TO DISPLACE 1 CU. MM.			
	Homogenized with gelatin	Gelatin added immediately after homogenization	Gelatin added just before freezing	No gelatin added
January 15.....	5.00	5.00	8.00	
January 29.....	2.40	3.00	3.00	
March 4.....	9.16	8.80	11.00	9.00 bottom (4.30 top)
March 11.....	7.50	10.00	10.00	8.00 bottom (3.40 top)
March 18.....	6.00	6.60	6.00	
April 16.....	1.66	2.00	2.00	1.35
May 12.....	3.14	3.38	3.14	

The data, presented in table 3, give the rate of melting of the ice cream at room temperature. The addition of gelatin prior to homogenization as compared with adding gelatin immediately following homogenization had no effect upon resistance to melting, but it can be readily seen that ice cream without gelatin or to which gelatin was added just before freezing melted more rapidly, especially during the first hour.

The results of the determinations of the hardness of the ice cream at  $-17.8^{\circ}$  to  $-21.1^{\circ}\text{C.}$  given in table 4 show much discrepancy from batch to batch caused by variations in the overrun,

etc. The overruns and other conditions being similar for each freezing within a series permit comparisons within each batch. It can be seen that the hardness was not affected by the time of adding gelatin and that it was greatly influenced by the movement of syrup in the ice cream containing no gelatin, as shown by tests made on the upper and lower sides of the brick.

*Effect of homogenizing ice cream mixes with and without sugar*

In recent years the general practice has been to add sugar to the mix prior to homogenization, probably to insure pasteurization

TABLE 5

*The effect of homogenizing the ice cream mix with and without sugar on viscosity*

DATE	HOMOGENIZED WITHOUT SUGAR		HOMOGENIZED WITH SUGAR	
	Single-stage valve	Two-stage valve	Single-stage valve	Two-stage valve
	<i>Cp.</i>	<i>Cp.</i>	<i>Cp.</i>	<i>Cp.</i>
April 8.....	328		464	
April 16.....	694		764	
May 21.....	400		554	
July 2.....	666		953	
September 25.....	2,800	2,190	3,600	1,800
October 9.....	2,720	870	5,360	4,900
October 16.....	1,570	990	2,080	1,300
October 30.....	1,530	620	4,480	2,880

of the sugar and to facilitate its complete solution. Numerous experiments were conducted, similar to those just given for gelatin, to determine the effects on the mix and the finished ice cream of adding sugar prior to and following homogenization. Since high viscosities developed under certain conditions the two-stage valve was also used on the homogenizer in some trials to learn how much it altered the results of the tests. Some of the mixes were frozen at a commercial plant to permit comparison of results with those secured in the experimental freezers.

The data given in table 5 show that homogenization of mixes with sugar always produced greater viscosities than when the

sugar was added at a later time. The tests with the two-stage valve gave mixes of lower viscosity, but again the presence of sugar during homogenization increased the viscosity. Numerous other experiments were made which gave the same results, the difference in viscosities being great enough to be easily observed.

In table 6 are data showing the overrun obtained on these mixes. Very low overruns were usually obtained with the experimental freezers on the viscous mixes which had been homogenized with sugar. The overrun was increased by the use of the two-stage valve, but the easier whipping of mixes homogenized

TABLE 6

*The effect of homogenizing the ice cream mix with and without sugar on the per cent of overrun obtained*

DATE	HOMOGENIZED WITHOUT SUGAR		HOMOGENIZED WITH SUGAR	
	Single-stage valve	Two-stage valve	Single-stage valve	Two-stage valve
	per cent	per cent	per cent	per cent
April 8.....	87.0		48 0	
April 16.....	89 0		63 0	
July 2.....	90 2		61 6	
May 21.....	93 0		54.2	
September 25.....	76.4	97 0	61.2	81.5
October 9.....	71.1	98.3	44 1	69.4
October 16.....	89.8	92.8	67.3	97 0
October 30.....	64.2	91.7	38 3	67.9

without sugar was still evident. On account of the impossibility of securing proper overrun in the experimental freezers without drawing the ice cream at too warm a temperature, some of the mixes were frozen at a commercial plant. It was found that in most cases an overrun approximating 90 per cent could be obtained, but the amount of whipping required after the brine had been turned off was variable. Thus the mixes homogenized without sugar whipped to the desired overrun in two minutes less time than when sugar was present during homogenization. A complete mix homogenized with the two-stage valve whipped in two minutes less time than the same mix homogenized with

TABLE 7

*The effect of homogenizing the ice cream mix with and without sugar on the size and clumping of the fat globules*

DATE	HOMOGENIZED WITHOUT SUGAR			HOMOGENIZED WITH SUGAR		
	Average size of fat globules in microns	Number of clumps found per 100 individual globules	Average size of clumps in microns	Average size of fat globules in microns	Number of clumps found per 100 individual globules	Average size of clumps in microns
Single-stage valve before freezing						
April 8.....	1.74	23	4 87 x 3.10	2.60	56	7.40 x 5.25
April 16.....	1.32	52	4 00 x 2.90	1.77	92	6.70 x 4.90
May 21.....	1.56	23	8 36 x 5.19	1.75	43	10.19 x 6.62
July 2.....	1.72	36	4 88 x 2.70	2.04	55	6.90 x 4.12
September 25.....	1.35	92	7 90 x 5.00	1.75	77	8.80 x 5.10
October 9.....	2.00	61	6 90 x 3.55	1.64	137	9.50 x 6.20
October 16.....	1.41	98	5 40 x 2.88	1.45	150	6.80 x 3.74
October 30.....	1.30	131	4 84 x 2.80	1.75	132	7.50 x 4.84
Average.....	1.55	64.5	5.89 x 3.51	1.84	92.5	7.97 x 5.09
Single-stage valve after freezing						
April 8.....	1.47	22	3 27 x 2.5	2.58	69	4.45 x 3.15
April 16.....	1.35	35	3 00 x 2.2	1.40	95	3.23 x 3.20
May 21.....	2.05	31	8 22 x 5.48	1.77	79	5.60 x 3.65
July 2.....	1.84	40	2 75 x 1.95	1.54	53	4.14 x 3.00
Average.....	1.67	32	4.31 x 3.03	1.82	74	4.35 x 3.25
Two-stage valve before freezing						
September 25.....	1.34	64	5.90 x 3.70	1.50	58	5.75 x 3.34
September 30.....	1.43	138	4.50 x 2.57	1.43	66	5.25 x 3.30
October 9.....	1.05	80	3.50 x 2.20	1.30	113	6.65 x 3.67
October 16.....	1.08	52	2.49 x 1.55	1.15	117	3.47 x 2.12
October 30.....	1.00	67	2.55 x 1.60	1.15	157	4.65 x 2.90
Average.....	1.18	80	3.78 x 2.32	1.30	102	5.15 x 3.06

the single-stage valve. Several dozen tests not reported in the table confirm the result that the mixes of high viscosity produced by the presence of sugar during homogenization were hard to whip.

An extensive study was made of the size of the fat globules and of the number and size of fat globule clusters in the mixes homogenized with and without sugar for the purpose of determining any relationships that might exist between the dispersion of the fat and the viscosity and whipping properties of the mix. A small portion of the data secured has been presented in table 7 which permits direct comparisons with the data on overrun and viscosity given in tables 5 and 6. The size of the individual fat globules was not affected by the presence of sugar during homogenization or by freezing the ice cream. The number of fat clusters and their size was increased by adding sugar prior to homogenization and their size was reduced by the freezing process. The two-stage valve produced slightly smaller individual globules and clumps than the single-stage valve. It is evident, therefore, that in a general way an increase in the size of fat clusters was associated with increased viscosity and difficult whipping, although it should be observed that the comparison should be made for the same mix homogenized with and without sugar. These results are in agreement with those of Mortensen (6) on the influence of homogenization on fat clumping and viscosity, and those of Martin and Dahle (7) on the influence of the two-stage valve on fat clumping, viscosity, and whipping.

The extensive data secured were too bulky to present in full. Measurement of 1235 individual globules of homogenized mixes without sugar with the single-stage valve gave an average size of 1.55 microns as compared with a size of 1.72 microns obtained for 1327 measurements of fat globules in the mix homogenized with sugar. The size of the fat clusters increased 1.69 x 1.11 microns when the mix was homogenized with sugar when compared with the mix homogenized without sugar, as shown by a total of 2056 measurements. The size of the fat clusters was reduced about 40 per cent due to freezing the mix, according to the average of 3405 measurements. The decrease in the size of fat clusters due to the

use of the two-stage valve approximated 30 per cent as shown by 983 measurements. The reduction in size of fat clusters due to freezing and the use of the two-stage valve was slightly greater when the mix contained sugar during homogenization.

The ice cream was judged by two judges in all of the experiments, comparing the homogenization of the mixes with and without sugar and with both the single-stage and two-stage valve. The texture and quality of the ice cream was not affected greatly by the addition of sugar to the mix before or after homogenization. The ice cream frozen from the mixes homogenized without sugar

TABLE 8

*The effect of homogenizing the mix with and without sugar on the hardness of the ice cream*

DATE	HOMOGENIZED WITHOUT SUGAR		HOMOGENIZED WITH SUGAR	
	Overrun	Grams to displace 1 cu. mm.	Overrun	Grams to displace 1 cu. mm.
	<i>per cent</i>		<i>per cent</i>	
August 20.....	90 0	1 20	90 0	1.33
September 3.....	95 0	1 60	88 0	1 77
October 2.....	70 3	3 38	74 4	4 00
October 16.....	92 8	2.17	97.0	2.17

was in most cases slightly smoother in texture than the ice cream frozen from the mixes homogenized with sugar.

Although considerable data were obtained upon the hardness and rate of melting of ice cream as influenced by the presence of sugar during homogenization and the use of the two-stage valve, they are not presented because of the difficulty in securing uniformity in the freezing process, particularly overrun. It is possible to select four batches reported in table 8 in which comparable yields were secured and which show uniform hardness and rate of melting, even though the treatment of the mixes varied as stated.

# INFLUENCE OF HOMOGENIZATION OF THE MIXES BEFORE AND AFTER CONDENSING

The fact that the mixes with less clumping and smaller sized clumps whipped easier and that the texture of the ice cream was improved made it desirable to endeavor to homogenize the ice cream mix in such a way, if possible, that there would be fewer and smaller clumps.

When milk which contained from 5 to 10 per cent of fat was homogenized, very little clumping of the fat globules was noticeable. These results furnished a clue for a method of preparing and homogenizing ice cream mixes to decrease the number and size of the fat globule clumps. Mojonnier and Troy (8) and Peterson and Tracy (9) recommended condensing the ice cream mix before homogenization.

Milk standardized to contain 10 per cent of fat was pasteurized and homogenized and sufficient water was removed from this mix by evaporation in partial vacuum so that when sugar, gelatin, and water were added it would contain the right proportion of ingredients. The size of the fat globule clumps in the mixes prepared in this way were greatly reduced and the average size of the fat clumps in the mixes after freezing were unusually small. There were a number of large individual fat globules with a few very small globules attached to them, apparently caused by fusion of some fat globules during condensing. These mixes whipped very readily. A mix prepared in this way frozen in a factory freezer was drawn from the freezer in seven minutes at a temperature of  $-3.4^{\circ}\text{C}$ . ( $25.8^{\circ}\text{F}$ .) with an overrun of 94 per cent. This ice cream was of very good quality.

In the next five experiments half of the mix was homogenized and then condensed, while the other half of the mix was condensed first and then homogenized. In these experiments the mixes which were homogenized before condensing whipped very readily, to an average overrun of 95.5 per cent, but the mixes which were condensed and then homogenized whipped to an average overrun of only 56 per cent. The addition of sugar to the 10 per cent milk homogenized before condensing did not make the resulting mix difficult to whip.

The ice cream frozen from the mixes homogenized before condensing was judged in comparison with the ice cream frozen from the mixes homogenized after condensing. The ice cream resulting from the first procedure was placed first in every case. The texture of this ice cream was somewhat better. Likewise its body was usually more creamy.

#### SUMMARY

The qualities of ice cream or the characteristics of the mix were altered but slightly by adding gelatin before or after homogenization. The fat clumps may have been larger when gelatin was added before homogenization, although the difference, if any, was small. The viscosity of the mix was slightly increased by adding the gelatin immediately after homogenization, but the whipping properties of the mix were not affected.

The texture of the ice cream was very slightly, yet noticeably, improved when the gelatin was added immediately after homogenization. Neither the melting resistance nor the hardness of ice cream varied with the time of adding the gelatin.

The data on the increased viscosity of the mix and on the improved texture of ice cream due to adding the gelatin after homogenization warrant the conclusion that the action of gelatin is slightly greater when added at this time. It is doubtful, however, that this difference is enough to be of commercial importance to the ice cream manufacturer.

The addition of sugar prior to homogenization greatly increased the extent of fat clumping, the viscosity of the mix, and the difficulty of securing the desired overrun.

The quality and properties of ice cream were not materially affected by the addition of sugar before or after homogenization, although in most cases the ice cream made from the mix homogenized without sugar was slightly smoother and more creamy.

The two-stage valve reduced the size of fat clusters, the viscosity of the mix, and permitted an easier incorporation of air.

The freezing process reduced the size of the fat clusters, but the reduction was not uniform for all mixes.



The mix homogenized before condensing contained smaller fat globule clumps, was easier to whip, and produced an ice cream slightly better in texture and quality than a similar mix homogenized after condensing.

The author wishes to express his appreciation to the Geneva Division of the General Ice Cream Company for the privilege of freezing some of the mixes at the Geneva plant.

#### REFERENCES

- (1) PRICE, W. V. 1925 An algebraic method of proportioning ice cream mixes. *Jour. Dairy Sci.*, ix, no. 2, 243-250.
- (2) PERKINS, A. E. 1914 An apparatus and method for determining the hardness of butterfat. *Jour. Ind. and Eng. Chem.*, vi, no. 2, 136-141.
- (3) HOLDAWAY, C. W., AND REYNOLDS, R. R. 1916 Effects of binders upon the melting and hardness of ice cream. *Virginia Agr. Exp. Sta. Bul.* 211, pp. 7.
- (4) DOWNEY, T. B. 1925 Using edible gelatin in ice cream. *The Ice Cream Trade Jour.*, xxi, no. 11, 81-84.
- (5) AMBROSE, A. S. 1926 Wisconsin hears the technical side. *The Ice Cream Trade Jour.*, xxii, no. 2, 59-60.
- (6) MORTENSEN, M. 1924 Some factors responsible for a desirable texture. *The Ice Cream Trade Jour.*, xx, no. 4, 74-76.
- (7) MARTIN, W. H., AND DAHLE, C. D. 1925 The use of the two-stage valve. *Penn. Agr. Exp. Sta. 38th Ann. Rpt.*, pp. 23-25.
- (8) MOJONNIER, T., AND TROY, H. C. 1922 *The Technical Control of Dairy Products*. P. 302. Mojonnier Brothers Company, Chicago, Ill.
- (9) PETERSON, R. W., AND TRACY, P. H. 1922 The condensation process of preparing and ice cream mix. *Jour. Dairy Sci.*, v, no. 2, 273-282.

# THE EFFECT OF HYDROGEN ION CONCENTRATION ON THE BACTERIAL CONTENT OF GELATIN\*

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## INTRODUCTION

The consuming public is becoming increasingly exacting in its demand for ice cream of high sanitary quality. The modern ice cream maker not only prides himself in the scrupulous care used in the handling of the mix in his plant, but he is also very fastidious in the selection only of raw products which are above reproach from the sanitary point of view. To this end the bacterial count has been employed as an index to the care used in the production and handling of the various ingredients of the ice cream mix. In many ice cream plants the sanitary quality of the gelatin is judged on a basis of the number of bacteria it contains. The tendency to associate high bacterial counts with insanitary conditions of manufacture and handling of gelatin comes largely through analogy with the bacterial analysis of dairy products.

The gelatin industry has made a tremendous improvement in the sanitary control of its manufacturing process during the past few years. It would be difficult to find an example of an industry that has done as much in as short a time toward the improvement of the sanitary quality of its product as has been accomplished in the past four or five years by the gelatin manufacturers. The recent article by Fay and Olson (1) published in 1927 was based on samples of gelatin collected in 1924. In this report the bacterial counts ranged from less than ten to 108,000,000 per gram of dry gelatin. Over half of the 50 samples analyzed contained in excess of 1,500,000 per gram. Bacterial analyses on samples collected after 1924 show that it is quite rare to find gelatin with such excessive numbers of bacteria.

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The question has been raised as to whether or not the bacterial count as applied to gelatin offers a satisfactory index to the sanitary quality of the product. It is ordinarily assumed that gelatin made and handled under carefully controlled conditions will have relatively few bacteria in it. On the other hand, gelatin which has been less carefully controlled may have a correspondingly larger number of bacteria. If a bacterial count has any value as one of the criteria of quality in gelatin it would be on the basis of the assumptions mentioned.

One difficulty that is frequently encountered when bacterial numbers are used as an index to quality, is the tendency to misinterpret the significance of the results of the analysis. The misinterpretation of bacterial counts is traceable in many instances to an attempt to use the results as an index to the safety of the product. The interpretative value of any bacterial count lies chiefly in its use as an index to laxity in sanitary control methods. If experience proves that it is feasible and practical to produce gelatin with less than a given number of bacteria per gram, a gelatin manufacturer who cannot meet this requirement, evidently is not exercising as much care in manufacturing and handling the product as he should. The extra 5000 or 100,000 bacteria in his gelatin may be, and in most cases are, perfectly harmless. The bacteria in themselves are of secondary importance; it is rather the conditions which their presence reveals that is of serious concern. It is only to the degree that laxity of control may pave the way for the entrance of pathogenic bacteria that the bacterial count has a connection with the safety of the product. The extent to which the laxity of control is a predisposing factor to contamination with pathogenic bacteria could be determined only by inspection.

In the manufacture of gelatin the practice is sometimes followed of adjusting the reaction to a rather high acidity (low pH) during the manufacturing operations. The pH may or may not be again adjusted to near the neutral point. Recently it has been claimed that the acidity of many gelatins is so high that bacterial development is inhibited. Sommer (2), Turnbow and Milner (3) and also Dahlberg (4) have shown that the value of

the keeping quality test is greatly diminished by the fact that the growth of liquefying bacteria, even though present in gelatin, may be inhibited by the high acidity of the product.

The question then arises as to whether the high acidity might not also render the bacterial count of doubtful value as a means of judging the sanitary quality of gelatin.

#### EXPERIMENTAL

With these points in mind the following experiment was designed to study the effect of the hydrogen ion concentration on the growth of microorganisms in gelatin. The 34 samples of gelatin used in the experiment were collected during the summer of 1927 from various American producers and distributors.

A 10 per cent solution of each of the samples of gelatin was prepared aseptically and 10 cc. placed in each of four sterile test tubes. Five drops of a 0.04 per cent aqueous solution of brom-thymol blue were added to each tube. The four tubes were divided into two pairs and labelled A and B, and X and Y. The X and Y tubes were each inoculated with 0.1 cc. of a mixed microbial suspension, in order to be certain that organisms were present. Preliminary plating of these gelatins showed that some were almost sterile, so that it was necessary to have a pair of tubes artificially contaminated. The organisms used for inoculating were obtained from an agar plate which had been exposed to the air and then incubated twenty-four hours. It was estimated that the 0.1 cc. inoculum introduced approximately 150,000 organisms, including yeasts, molds, spore-bearing and non spore-bearing bacteria.

Tubes A and B were not inoculated, and any subsequent microbial development was dependent upon the original flora of the gelatin.

In one tube of each pair, tubes B and Y, the reaction was adjusted to pH 7.0, and in the remaining tube of each pair, tubes A and X, the reaction of the original gelatin was not altered. Adjustment of the reaction to pH 7.0 was accomplished by admitting sterile NaOH until the brom-thymol-blue approached a grass-green color.

The set of four tubes, therefore, consisted of two uninoculated and two inoculated tubes of gelatin, one tube of each pair having the reaction of the original gelatin unaltered, and the remaining tube of each pair with the reaction adjusted to pH 7.0.

The aim of this experiment was to determine the effect of the hydrogen ion concentration of the gelatin on the growth of microorganisms. If no growth developed in tube A (the original gelatin) it might have been the result of one or more of the following factors; sterility, reaction, or the presence of antiseptic substances. The inoculated tubes, X and Y, would serve as controls on sterility. If failure of growth in tube A were due to the second factor, viz., the reaction, tubes B and Y with a neutral reaction would serve as checks. If lack of growth in tube A were due to the combined effect of the two factors, sterility and reaction, tube Y would be the only one in the series in which growth would obtain. If an antiseptic or disinfectant substance had been added, there would probably be no growth in any of the tubes.

All tubes were incubated twenty-one days at 37°C., and the number of days required for growth to become evident was recorded. No growth after twenty-one days was regarded as negative. All samples showing no growth after twenty-one days were examined microscopically, and subcultures were made by transferring 0.1 cc. of the gelatin from the incubated tube to sterile broth. If the failure of growth in the gelatin were due to antiseptic action, removal of organisms that were still viable would likely result in their development in the broth subculture.

In table 1 are shown the bacterial count per gram of dry gelatin, the pH, the keeping quality in days at room temperature, the price per pound, and the time required for growth to appear in the four tubes treated in accordance with the previous description.

The pH values were determined by the colorimetric method of Medalia (5) and the plate counts were made in accordance with the Standard Methods of the American Public Health Association.

Referring to the data in columns A, B, X and Y in table 1, it

TABLE 1

*The results of bacterial count, reaction and keeping quality studies with 34 samples of gelatin*

SAMPLE NUM- BER	BACTERIAL COUNT PER GRAM	pH	KEEPING QUALITY		GROWTH				GROWTH IN SUBCUL- TURES FROM NEGATIVE TUBES	PRICE PER POUND
			Colo- nies appear- ed	Surface lique- faction	In uninocu- lated tubes		In inoculated tubes			
					A	B	X	Y		
					pH un- altered	pH ad- justed to 7.0	pH un- altered	pH ad- justed to 7.0		
			days	days	days	days	days	days		cents
16	900	4.8	3	—	7	2	5	2		50
15	40	4.9	—*	—	6	4	5	2		55
17	100	4.9	3	—	7	3	5	1		45
18	4,500	5.1	4	20	13	2	11	1		40
23	1,200	5.3	9	17	—	2	9	2	+	40
22	900	5.5	3	—	10	2	10	1		35
9	12,000	5.6	7	13	13	3	5	1		27.5
19	25	5.7	3	20	5	2	2	1		43
25	200	5.7	9	18	—	5	5	1	+	35
7	250	5.7	6	10	2	2	2	1		55
20	2,500	5.7	4	—	4	2	2	1		50
6	Less than 5	5.8	21	—	—	5	5	2	+	55
8	10	5.8	7	18	10	4	5	2		45
21	800	5.8	3	13	4	2	1	1		40
33	5	5.9	—	—	—	6	5	5	—	45
12	60	5.9	3	7	2	2	2	1		25
34	30	6.2	13	17	—	—	2	2	++	50
2	5	6.3	3	7	5	2	1	1		44
10	5	6.3	7	—	10	10	1	2		54
32	10	6.3	—	—	—	7	5	7	—	40
24	Less than 5	6.4	6	10	5	4	2	1		55
14	10	6.4	10	18	9	9	1	2		38
4	Less than 5	6.5	4	9	2	3	1	1		57
5	Less than 5	6.5	—	—	16	5	1	1		50
13	10	6.5	10	21	13	4	2	2		45
11	20	6.5	4	7	6	5	1	1		34
1	4,900	6.5	4	9	3	3	1	1		32
28	9,500	6.5	6	9	7	4	1	1		39
3	15,000	6.5	3	—	2	2	1	1		39
27	20,000	6.5	1	2	1	2	1	1		30
26	80	6.6	9	13	5	5	1	1		45
30	170	6.6	7	—	—	6	5	6	—	51
31	80	6.6	6	20	7	—	2	5	—	56
29	1,100	6.6	—	—	—	6	21	5	—	44

\* The minus sign (—) indicates no growth in 21 days incubation.

will be noted that organisms failed to develop in one or more of the tubes prepared from samples 23, 25, 6, 29, 30, 31, 32, 33, and 34. Samples 29, 30, and 31 were obtained from the same company, 32, 33, and 34 were from another company, and each of the others, 6, 23, and 25 were from separate manufacturers.

Sample 23 showed no growth in tube A in twenty-one days, but when the reaction was adjusted to the neutral point, as in tube B, growth was evident in two days. The subculture made from tube A after twenty-one days revealed the presence of living organisms and that microbial growth in the original gelatin had been inhibited. Heavy inoculation of this gelatin, as in tube X, resulted in growth after nine days in spite of the reaction. It is evident that in this sample of gelatin, the reaction has been a determining factor in keeping down bacterial development. What has been said about sample 23 applies equally well to samples 25 and 6.

In the case of samples 30, 32, and 33, bacterial growth not only failed to develop in the original gelatin (tube A) in twenty-one days, but there was also no growth in subcultures made from these tubes. Sample 34 showed no growth in tubes A and B, but growth was obtained in the subcultures. Considering the low bacterial counts of these gelatins, it seems logical to attribute the sterility of tubes A and B to the absence of organisms capable of developing in a gelatin medium. In sample 31 the failure of growth in tube B (pH 7.0) is probably the result of the presence only of acidophilic types.

The results obtained with sample 29 are not so easy to explain. It will be noted that tube A showed no growth after twenty-one days and also no growth in the subculture. The mixed culture of organisms inoculated into tube X, however, failed to show any evidence of growth until the twenty-first day of incubation. When the reaction was adjusted to pH 7.0, growth occurred in less than one week. One might assume that the reaction was the inhibitory agent, until it is noted that the reaction of the original gelatin was almost neutral (pH 6.6). The probability that there was a disinfectant present is virtually eliminated by the readiness with which growth appeared in tubes B and Y.

The bacterial count showed 1100 per gram for this gelatin, which would not justify attributing the lack of growth in tube A to the relative sterility. It seems evident that the factor which prevented bacterial development was effective in a slightly acid medium, but less effective in a neutral gelatin. Repetition of the work with this sample has given essentially the same results.

The results of these experiments indicate that although the acidity of many of the gelatins studied had a retarding effect on the growth of bacteria, it did not entirely prevent their development. The adjustment of the reaction to pH 7.0 hastened growth in 21 of the 34 gelatins, especially in those with a high acidity.

The effect of neutralizing the gelatin is particularly noticeable in the results obtained with the inoculated tubes X and Y. With but three exceptions (7, 20, and 21) an original reaction more acid than pH 5.9 in tube X required more than twice as long for growth to become evident, than was required in tube Y having a neutral reaction. In table 1 it may also be observed that in only one of the samples (no. 29), having a reaction less acid than pH 5.9, did neutralization of the gelatin in tube Y produce any marked effect on the rate of microbial development. However, several of the samples more nearly neutral than pH 5.9 showed more rapid development of organisms in tube B than in tube A. Failure for growth to develop in several of the A and B tubes was very likely due to the fact that there were very few organisms present.

It is a well established fact that many microorganisms are more easily killed in a high acid medium than in one that is neutral in reaction. The practice of manufacturing gelatin with the reaction at a low pH takes advantage of this fact and results in more effective bacterial destruction. Furthermore, during the drying process there is much less likelihood of bacterial growth taking place if the reaction is very acid. When gelatin is placed in the drying tunnels, heavy contamination from the air may result, unless special precautions are taken to purify the air. The results of this experiment indicate that very little growth would take place during the drying process if the gelatin had a



low pH, whereas growth would not be inhibited if the reaction were near the neutral point. The sanitary conditions being equal, one would expect a gelatin which had been processed with a high acidity to contain fewer bacteria than one processed with the reaction near the neutral point. In other words, the reaction at which a gelatin has been processed should be taken into consideration when the bacterial count is used as an index to the sanitary conditions surrounding the production of the gelatin.

On the other hand, one would not necessarily expect a correlation between the reaction and the bacterial count. In view of the fact that some manufacturers readjust the reaction of their gelatin to near the neutral point, the pH of the finished product may not reveal the reaction at which it was processed. A survey of the bacterial counts and the pH values given in table 1 at once

TABLE 2  
*The distribution of pH values of 34 samples of gelatin*

	pH				
	4.8-5.0	5.1-5.5	5.6-6.0	6.1-6.5	6.6
Per cent of samples.....	8.8	8.8	29.4	41.2	11.8

shows this lack of correlation. Tracy and his coworkers (6) in reporting a study of 48 samples of gelatin, state that most of the samples with a low pH also had a low bacterial count. The reactions of the samples analyzed by them ranged from pH 4.05 to 7.35.

The reaction of most of the samples was between pH 5.5 and 6.5. Table 2 shows a distribution of the samples based on the reaction. There were 8.8 per cent of the samples with a reaction between pH 4.8 and 5.0; 8.8 per cent between pH 5.1 and 5.5; 29.4 per cent between pH 5.6 and 6.0; 41.2 per cent between pH 6.1 and 6.5; and 11.8 per cent with a reaction of pH 6.6.

#### KEEPING QUALITY TEST

The results of the keeping quality test are recorded in table 1 under two headings; the days required for the appearance of

colonies, and for the first evidence of surface liquefaction of the gelatin. It will be noted that 5 of the 34 samples (14.7 per cent) showed no colony development after twenty-one days at room temperature, and 13 samples (38.2 per cent) did not show any liquefaction after the same period of incubation. There were 9 other samples which required more than two weeks for liquefaction to appear. That liquefaction is not necessarily associated with high bacterial counts may be seen by noting the results with samples 19, 8, 12, 34, 2, 24, 14, 4, 13, 11, 26, and 31, each of which showed liquefaction in from one to three weeks, even though the bacterial count did not exceed 80 per gram in

TABLE 3

*The increase in the per cent of samples of gelatin having a low bacterial content, based on samples collected in 1924 and 1927*

PLATE COUNT PER GRAM OF GELATIN	34 SAMPLES COLLECTED 1927	50 SAMPLES COLLECTED 1924
	per cent	per cent
Sterile (1 to 5 dilution)	12	0
10 or less	32	2
100 or less	56	4
1000 or less	73	14
5000 or less	88	20
10,000 or less	91	22
20,000 or less	100	30

any case. Two of these samples (nos. 24 and 4) liquefied on the surface in ten and nine days respectively, although the bacterial analysis in each case resulted in sterile plates in 1 to 5 dilutions. Molds were responsible for liquefaction of most of the samples. It would seem logical from the experience with these gelatins that a mold count would check more closely with the liquefaction test than a bacterial count.

#### IMPROVEMENT IN SANITARY QUALITY OF GELATIN

At various places in the literature statements are made to the effect that the bacterial content of gelatin now on the market is very much lower than it was a few years ago. Evidence to support this idea is afforded in the results in table 3. The bac-

terial counts of 50 samples of gelatin collected in 1924 by Fay and Olson (1) are compared with the bacterial counts of the samples reported in this paper. Table 3 shows a comparison of the percentage of samples having less than a given number of bacteria per gram in the groups of gelatins collected in 1924 and 1927 respectively. It may be noted, for example, that only 4 per cent of the samples collected in 1924 contained less than 100 bacteria per gram, whereas in 1927, 56 per cent contained less than this number. Similarly, in 1924 only 30 per cent of the gelatins studied contained 20,000 or less bacteria per gram, and in 1927 none of the samples exceeded this figure. It is believed that the samples are sufficiently representative of the gelatins now in use in ice cream plants of this country to justify the assertion that the bacterial content of gelatin now available to the ice cream industry has been greatly reduced from what it was a few years go.

#### SUMMARY

A study of 34 samples of gelatin failed to reveal any relation between the reaction and the bacterial count. The bacterial counts ranged from less than 5 bacteria per gram to 20,000, and the reactions expressed as pH were between 4.8 and 6.6. The keeping quality test failed to check with the relative bacterial counts since some of the samples with the highest counts failed to liquefy in twenty-one days at room temperature, and some of the apparently sterile samples developed liquefaction in as short a time as nine days.

A study of the effect of the reaction on bacterial growth revealed that the high acidity of some of the samples had a marked deterring action on the rate of growth of the microorganisms. Although the high acidity of some of the gelatins exerted a marked retarding effect on the development of microorganisms, in no case did the reaction completely inhibit growth where a mixed microörganic population was added.

Under practical conditions, gelatin produced in an insanitary plant where no effort is made to prevent contamination, especially during the drying process, the resulting contamination would

likely be composed of a wide variety of microorganisms, comparable perhaps to the heterogeneous mixture used in artificially contaminating the gelatins in this experiment. The results obtained indicate that if such contamination were to take place while the gelatin was in the drying alleys, very little growth would be likely to result in those gelatins having a high acidity. In other words, the resulting microbial numbers would approximate the extent of contamination since little or no growth would have taken place. On the other hand, if a gelatin with a nearly neutral reaction were subjected to the same contamination, growth of the organisms during the drying process would likely result. A numerical estimate of the microorganisms in this instance would lead to a misinterpretation of the relative sanitary conditions under which the two gelatins were produced.

Undoubtedly many of the organisms present in the finished product die before the gelatin reaches the consumer as a result of the unfavorable environmental conditions, of which high acidity is only one. In other words, the bacterial analysis, under any conditions, is likely to present a picture of the conditions surrounding production of the gelatin which has been modified by the effect of environmental factors on the microbial flora of the finished product. Even though some of the more sensitive organisms originally present in gelatin may have been destroyed, and the growth of other microorganisms deterred by adjustment of the pH, it is believed that gelatin which has been carelessly produced will have a relatively high bacterial count. However, intelligent interpretation of any bacterial count, whether it be on gelatin or any other product, depends on a thorough understanding of, and adequate allowances for, all the factors which may tend to increase or decrease the number of bacteria. The results of this investigation indicate that in interpreting a low bacterial count of gelatin, one of the factors to be taken into consideration is the reaction. This, however, does not render the bacterial count of gelatin useless. The purchaser of gelatin can eliminate from consideration many samples of gelatin because of the undesirable conditions which their high bacterial counts reveal. He cannot, however, be absolutely certain that gelatin

with a low bacterial count is *per se* beyond reproach from the standpoint of sanitary quality. This interpretation never should be made on the basis of a bacterial analysis of any product. A bacterial count on gelatin or any other product only indicates a conclusion and never proves it, unless that conclusion is corroborated by an inspection of the source of supply. A low bacterial count indicates that at least an effort has been made on the part of the manufacturer to prevent excessive contamination of his product and to destroy those bacteria which do gain entrance. If the acidity is high, it is likely that the low bacterial count may be due in part to the death of organisms incapable of surviving the environment, and to the increased efficiency of bacterial destruction by heat in high acid media. Nevertheless, if gelatin has a low bacterial count it indicates, even though it does not prove, that the conditions under which the gelatin was produced did not permit of excessive contamination or else more of the resultant heterogeneous microbial flora would have survived. A low bacterial count is, therefore, believed to be a fairly good index to gelatin of good sanitary quality, although the factors affecting bacterial destruction and growth, such as the acidity, must be taken into consideration.

#### REFERENCES

- (1) FAY, A. C., AND N. E. OLSON 1927 The effect of gelatin on the bacterial content of ice cream mix. Jour. Bact., xiv, 5, 363-376.
- (2) SOMMER, H. H. 1927 Gelatin and mix viscosity. Ice cream Trade Jour., xxiii, 1, 47-48.
- (3) TURNBOW, G. D., AND F. W. MILNER 1927 The rôle of gelatin in ice cream. Jour. Dairy Sci., x, 3, 202-209.
- (4) DAHLBERG, A. C. 1927 Some new facts about gelatin and its use in ice cream. 1927 Twenty-seventh Ann. Rept. Int. Assn. of Ice Cream Mfgs., 124-128.
- (5) MEDALIA, LEON S. 1920 "Color standards" for the colorimetric measurement of H-ion concentration pH 1.2 to pH 9.8. Jour. Bact., v, 5, 441-468.
- (6) TRACY, P. H., H. A. RUEBE, AND J. M. BRANNON 1926-1927 Ann. Rept. Ill. Agr. Exp. Sta. 162.

# A COMPARISON OF THE VOLATILE-SOLVENT METHOD WITH THE VACUUM-OVEN METHOD FOR DETERMINING MOISTURE CONTENT OF CHEESE\*

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In the work described in this paper the writer has added improvements to the technique of the volatile solvent (toluene) method, also known as the distillation method, of moisture determination, and has compared this method with the vacuum-oven method at 98° to 100°C., in determinations on a number of varieties of cheese.

The distillation method of moisture determination was first used by Marcusson (1) in 1905, and has since been improved by Rogers (2), Michel (3), Hart (4), Dean and Stark (5), Bidwell and Sterling (6), Normann (7), Gisiger (8), and Jones and McLachlan (11). It has been tentatively adopted for certain organic substances by the Association of Official Agricultural Chemists (9, 10).

This method consists essentially in boiling a weighed sample of cheese with toluene (boiling point, 111.0°C.) or other suitable volatile solvent in a flask connected to a condenser by means of a Bidwell and Sterling (6) distilling tube receiver, and calculating the moisture content by reading, on the graduated portion of the tube, the amount of moisture which has distilled over. The method is fully described by the investigators mentioned above.

A few important details of the method, however, should be mentioned. All moisture adhering to the upper part of the distilling tube is pushed down, after the tube is disconnected, by the use of a rubber policeman described by Rogers (2). The

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rubber should be small and should be cut to a rather thin and narrow edge. A small copper wire with a loop on the end, described by Hart (4), should be used for brushing free any droplets of water or toluene which adhere to the surface of the smaller part of the tube.

Before being used, each tube is numbered and accurately calibrated by running several check tests on accurately measured quantities of water, as described by Bidwell and Sterling (6). These investigators obtained readings of 1.98 cc. when using 2 cc. of water, thus reading the tube with a correction of +0.02 cc.

Improvements have been added to the technique of the method. The paraffin or glycerine bath in which the flasks are to be boiled, described by Rogers (2), is heated to a temperature of about 140°C. and this temperature is gradually increased to about 150°C. at the completion of the test. Thus the distillation is conducted at the rate of about two drops per second at first, to about three to four drops per second before boiling is stopped; and the flow of distilling liquid is brought to about 1 to 2.5 cm. above the lower end of the cold-water column of the condenser. The use of the bath prevents bumping in most cases and reduces charring of the sample to a minimum.

The corks are soaked in collodion and allowed to dry for at least an hour before being used, in order to prevent leakage or absorption of moisture by the corks.

The tubes are placed in a water bath at room temperature for an hour or longer before reading.

It is obvious that the percentage composition of a weighed sample must be measured in terms of weight rather than of volume. The correction for the density of water (0.012 cc. for 4 cc. at 20°C.) is subtracted from the corrected tube reading.

#### EXPERIMENTAL

A number of samples of Swiss cheese were boiled for one and one-half hours, the tubes removed, and the readings taken. The boiling was then continued for two hours, new tubes were used, and an average of 0.6 per cent additional liquid was distilled over. This last distillate was treated with  $\text{KMnO}_4$  and gave a

heavy precipitate of  $\text{MnO}_2$ , indicating that the last traces of distillate might be largely formic acid (boiling point,  $100.47^\circ\text{C}.$ ) or rather aldehyde or acid impurities. In fact the distillate from routine tests of cheese at one and one-half hours or longer, when treated with permanganate, fades slowly in color from purple to cherry red, and finally a trace of precipitate settles out. This indicates the presence of impurities and may partially

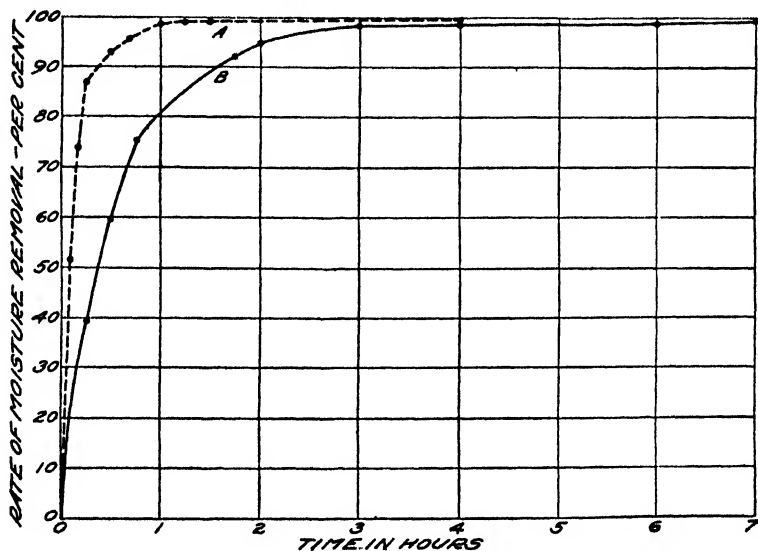


FIG. 1. SHOWING RATE OF REMOVAL OF MOISTURE FROM SWISS CHEESE BY THE TOLUENE-DISTILLATION METHOD AND BY THE VACUUM-OVEN METHOD AT  $98^\circ$  TO  $100^\circ\text{C}.$

A, distillation method; B, vacuum-oven method at  $98^\circ$  to  $100^\circ\text{C}.$

account for the fact that the readings obtained by the distillation method are slightly higher than those obtained by the vacuum-oven method.

Four samples of Swiss cheese were run in the vacuum-oven until they ceased to lose weight. The time required was twelve hours. They were then transferred quickly to the toluene flask and boiled for one hour, resulting in 0.78 per cent additional distillate. This liquid showed a trace of impurity by the permanganate test. A



TABLE 1

*Comparison of the distillation method and the vacuum-oven method for determining the moisture content of five varieties of cheese*

OVEN METHOD		TOLUENE METHOD		DIFFERENCE	OVEN METHOD		TOLUENE METHOD		DIFFERENCE
Domestic Swiss, cured					Domestic Swiss, green				
per cent	per cent (average)	per cent	per cent (average)	per cent	per cent	per cent (average)	per cent	per cent (average)	per cent
33.42		34.87			37.54		38.62	38.62	+1.02
33.40	33.41	34.69	34.78	+1.37	37.67	37.60			
34.27		34.88			36.11		36.90		
34.22	34.24	35.20	34.74	+0.50	35.89	36.00	36.30	36.60	+0.60
		34.50			37.46		38.55		
		34.40			37.30	37.38	38.35	38.45	+1.07
33.53		34.37			35.90		36.60		
33.57	33.55	34.53	34.20	+0.65	35.83	35.86	36.80	36.70	+0.84
		34.00			36.56		37.72		
		33.90			36.34	36.45	37.36	37.54	+1.09
34.35		35.46			36.87		37.82		
34.30	34.32	35.00	35.23	+0.91	36.76	36.81	37.72	37.87	+1.06
35.56		35.98			36.09		37.17		
35.35	35.45	36.26	36.12	+0.67	36.30	36.19	36.83	37.00	+0.81
32.32		33.54			Total average....	36.61		37.54	+0.93
32.36	32.34	32.72	33.13	+0.79	Cottage				
33.44		34.45			77.90		78.40		
33.51	33.47	34.65	34.55	+1.08	77.04	77.47	77.80	78.10	+0.63
35.16		35.40			Cheddar				
35.11	35.13	35.90	35.65	+0.52	34.63		35.70		
35.09		35.10			34.65	34.64	36.00	35.86	+1.22
35.04	35.06	35.60	35.35	+0.29			35.90		
Total average.....	34.11		34.86	+0.75	Roquefort				
Brick					41.35				
37.01		37.90			41.23	41.29	41.70	41.70	+0.41
37.14	37.07	38.20	38.05	+0.98	39.27		39.90		
Imported Swiss					39.13	39.20	39.50	39.70	+0.5
34.37		35.50			Total average....	40.24		40.70	+0.46
34.82	34.59	35.30	35.60	+1.01					
		35.70							
		35.90							

Average of all varieties +0.854

\* Determination on a different sample from the same cheese, ten days later, by another investigator.

uniform time of one and one-half hours for boiling was decided upon for the varieties of cheese studied.

All samples of Swiss cheese were taken with a cheese trier. These sample plugs were about 6 inches in length, but only the 3-inch portion nearest the center of the cheese was used. A vacuum of 24 inches and a constant temperature of 98° to 100°C. were maintained in the oven.

Figure 1 shows the results of a detailed study of the rate of removal of moisture from Swiss cheese. Curve B represents a total of 51 weighings taken at irregular intervals, considering the results obtained at ten hours as 100 per cent.

It was found that at least six to seven hours was required to dry the samples to constant weight in the oven and that after ten to twelve hours practically no further loss of weight occurred in ten hours additional boiling.

In Curve A the figures obtained upon the samples boiled for four hours were taken as 100 per cent, and no appreciable distillation was noted after four hours.

A comparison of the two methods on five varieties of cheese shows an increase in moisture content by the volatile-solvent method at one and one-half hours, ranging from 0.46 per cent for Roquefort cheese to 1.22 per cent for Cheddar cheese, when compared with the results obtained by the vacuum-oven method. These figures include the average tube correction, +0.4 per cent, and the deduction for water density, -0.1 to -0.12 per cent.

Closer checks were obtained by the vacuum-oven method than by the distillation method. Both methods produced a pronounced brown color in fresh cheese which still contained lactose, indicating that lactose decomposition was brought about by both methods. (See table 1.)

#### SUMMARY

Toluene removes moisture rapidly without causing undue charring or decomposition of the material and is recommended as a very satisfactory distilling liquid.

Coating the corks with collodion, correcting for the density of water, and using a paraffin or glycerine bath at 140° to 150°C.,

are improvements which increase the efficiency and accuracy of the distillation method.

This method gives results for cheese which are slightly higher than the results obtained by the vacuum-oven method. It is considered undesirable to boil the material more than one and one-half hours when the rate of boiling is properly regulated.

Closer checks are obtained on duplicate samples by the vacuum-oven method than by the distillation method. The latter method is much more rapid and is very accurate for determinations on cheese.

#### REFERENCES

- (1) MARCUSSON, J.: Die Bestimmung des Wassergehalts von Ölen, Fetten, Seifen, Harzen, usw. Mitt. kg. Materialprüfungsamt, xxiii, (1905) 58.
- (2) ROGERS, J. S.: Report on Tannin: Method for Greased Leather. Bull. 137, U. S. Dept. Agriculture, (1910) 172.
- (3) MICHEL, F.: Zur direkten Wasserbestimmung in Nahrungsmitteln und Gebrauchsgegenständen durch Destillation. Chem. Zeit., xxxvii, (1913) 353; Jour. Soc. Chem. Ind., xxxii, (1913) 445.
- (4) HART, R.: An improved distillation method for the determination of water in soap. Jour. Ind. Eng. Chem., x, (1918) 598.
- (5) DEAN, E. W., AND STARK, D. D.: A convenient method for the determination of water in petroleum and other organic emulsions. Jour. Ind. Eng. Chem., xii, (1920) 486.
- (6) BIDWELL, G. L., AND STERLING, W. F.: Preliminary notes on the direct determination of moisture. Jour. Assoc. Off. Agric. Chem., viii, (1925) 295.
- (7) NORMANN, W.: Zur Wasserbestimmung in Fetten und anderen Stoffen. Z. angew. Chem., xxxviii, (1925) 380; Chem. Abst., xix, (1925) 2277.
- (8) GISIGER, F.: Zur Wasserbestimmung durch Destillation. Mitt. Lebensm. Hyg., xvii, (1926) 4; Chem. Abst., xx, (1926) 2130.
- (9) Changes in official and tentative methods of analysis. V. Insecticides and fungicides. Jour. Assoc. Off. Agric. Chem., ix, (1926) 28.
- (10) Changes in the official and tentative methods of analysis. IX. Feeding stuffs. Jour. Assoc. Off. Agric. Chem., ix, (1926) 30.
- (11) JONES, J. M., AND McLACHLAN, T.: Volatile solvent method. The Analyst, lii, (1927) 383.

## BOOK REVIEWS

*Fundamentals of Dairy Science.* By Associates of Lore A. Rogers. The Chemical Catalog Company, New York, 1928.

"Fundamentals of Dairy Science" is the first book published in the English language that deals with dairy science as a science. Its publication is a recognition of the importance of dairy science and it should assist in promoting a better understanding of and the need of research in certain phases of this branch of science.

This book was written by "Associates of Lore A. Rogers" in the research laboratories of the Bureau of Dairy Industry, United States Department of Agriculture and dedicated to him in recognition of his contributions to pure science and its applications to industry. It is a valuable contribution to dairy science and its applications.

The book is divided into four parts which deal with "The Constituents of Milk," "The Physical Chemistry of Milk and its Products," "The Microbiology of Milk and Milk Products," "The Nutritive Value of Milk and Milk Products. The Physiology of Milk Secretion." It is noteworthy that the authors did not include studies of the breeding of dairy cattle and only a limited amount of material on feeding is presented as a part of the physiology of milk secretion.

The 28 authors of the book have written parts of or entire chapters treating subject material that has been in their special field of work. They present the status of our knowledge as given in the literature, in new unpublished material, and as critically interpreted by them. The extensiveness of the survey of existing literature is evident from the fact that 1332 references are given, yet some investigations that one might expect to find in this book are absent. Of necessity, there is much difference in the technicality and clearness of presentation due primarily to the variety of material presented, but to some extent to the styles of the different authors. It should be stated that those who edited the book were successful in correlating the contributions to make a uniform book and one which is surprisingly free from errors for so extensive a publication.

A reviewer would be extremely presumptuous to critically comment upon the material presented in a book of this character. One cannot be able to do more than accept a contribution on milk fat by Geo. E. Holm, on pigments of milk by L. S. Palmer, on acid-base equilibria by

W. M. Clark, on sources of bacteria in milk by J. M. Sherman, etc.; not to mention any of the various subjects presented by the other 24 authors. One can judge the book to best advantage by first reading those chapters dealing with work in which he has been active to learn if the subject has been completely and accurately presented, and by reading the contributions in which he wishes to gain more information to learn if it gives the knowledge desired. Considered in this manner the book is especially pleasing, not only to obtain the results of leading scientific investigations on a given subject but to gain an accurate, concise view of the subject as a whole.

A. C. DAHLBERG.

*Physik der Milchwirtschaft.* (Physics of the Milk Industry.) OTTO RAHN AND PAUL F. SHARP. Paul Parey, Berlin. 1928. 227 pages, 48 illustrations.

As stated by the authors in the preface, this is the first attempt to assemble into book form the mass of data dealing with the physics of milk. In view of the nature of the material presented, however, it would probably be more accurate to term the treatise "The physico-chemical aspects of milk and its products."

The data have been arranged so as to serve the technical as well as the research workers, hence there is necessarily a duplication of some of the material. For example—Chapters I-IX inclusive deal directly with the physical properties and various phenomena concerned with milk, cream and butter in terms of viscosity, milk foam, creaming, butter formation, butter structure, and water content of butter. Chapters X-XV deal with the following products: whipped cream, lactose, ice cream, casein, cheese, condensed milks, and dry milk. It is evident that the material in these chapters must necessarily duplicate some of the material in the preceding ones.

Presentation of the entire material from the standpoint of the various products, or from the standpoint of the fundamental physical properties concerned, would have led to a clearer and more concise outline. This criticism is, however, of a minor nature and should in no way militate against the value of the mass of excellent data presented.

The treatment of most of the subjects is thorough, especially that of cream rising, and butter formation and structure, and the data are well illustrated with tables and graphs. The literature citations are numerous and well chosen.

The book is an excellent contribution to the literature of dairy science.

GEO. E. HÖLM.

## STUDIES ON BUTTER SALTS\*

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### PURPOSE OF INVESTIGATION

The salt in butter is a factor in commercial butter manufacture which has been held responsible, in many instances, for diverse butter defects, such as defects in flavor, body, texture, and color. Instances have also come to our attention of creameries claiming to be able to incorporate more salt and to secure a larger overrun with certain brands of salts than with others.

Bitterness is one of the principal flavor defects that has been ascribed to the influence of salt and this is commonly associated in the practical mind with the presence of impurities in the salt. Magnesium and calcium chlorides, for instance, have a bitter character and salt manufacturers have endeavored to produce butter salts that are as free as possible from these and other chemical impurities.

The question of ease of salt incorporation and the presence or absence of free moisture in butter have led to various claims regarding the influence of individual brands of salt. These questions, together with the usual difficulties in maintaining a satisfactory overrun and in avoiding grittiness in butter, are confronting the practical buttermaker with real problems.

The color of butter is known to be influenced by the salt. The appearance of mottles, streaks, and waves, has suggested the possibility of a relation between unevenness of color and type of salt used.

The purpose of the present investigation, therefore, was to determine if, and to what extent, the type and brand of butter salt is a factor in those problems of the buttermaker that have to do with the control of the overrun and with guarding against certain butter defects.

\* Received for publication April 20, 1928.

## SCOPE OF INVESTIGATION

Ten well known butter salts were included in this study. They were obtained in standard barrels taken from commercial stock. These salts are listed in this report according to number from 1 to 10. Three brands were flake salts and the remaining 7 were cube salts. The flake salts are represented by the numbers 1, 2, and 3. The remaining numbers refer to cube salts. Each salt was studied in accordance with the following outline:

Physical, chemical, and bacteriological analyses  
Effect of salt on flavor, body, texture, and color of butter  
Effect of impurities in salt on flavor of butter  
Suitability for use in soaking parchment wrappers and liners

## SAMPLING FOR EXAMINATION AND ANALYSIS

All samples for physical and chemical analysis were taken into dry Mason jars from near the center of the barrel after about 50 pounds of the salt had been removed. Samples for bacteriological examination were taken into sterile 4-ounce bottles.

## PHYSICAL ANALYSIS

*Condition of salt in barrel.* Brands 4, 5, 6, and 8 were profusely caked, necessitating the use of a pick to loosen before removing with scoop. Brands no. 2, 3, 7, 9, and 10 showed slight caking into loose masses and lumps, but these broke down easily giving no handling difficulty. Brands no. 2 and 3 appeared damp and had little flowing tendency, while the other salts appeared dry and loose, and could be poured out like sugar.

*Color of dry salts.* Portions of the salt were placed on sheets of black paper and arranged in the order of their relative whiteness. Comparison was facilitated by pressing glass plates down on the piles of salt. The colors ranged from pure snow white to cream color. Nos. 3, 2, 10, 7, and 5, in the order given ranged from pure white to the first shade of cream. Nos. 1, 4, 9, 6, and 8, in the order given, showed increasingly marked creamy color.

*Bulkiness or relative density.* The bulkiness of the salts was determined, and is here expressed, in terms of relative density.

This was done by comparing the weight of a given volume of each salt with the weight of the same volume of water. For this purpose a 200 cc. assay flask was used. This flask was packed by uniform jarring against a solid surface. Checks within one gram were readily obtained between duplicates. The relative densities of the dry salt were as follows, using the density of water as 1.

Flake salts (nos. 1 to 3).....	0.82-0.90 (average 0.86)
Cube salts (nos. 4 to 10).....	1.23-1.28 (average 1.26)

The above figures show that the flake salts are of the lowest relative density, their weight being less than that of water. The

TABLE 1  
*Percentages of salt passing through screens of various mesh*

SALT NUMBER	MESHES TO THE INCH						
	Over 20	20	30	40	60	80	100
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	0.2	15.6	8.2	40.7	20.7	8.0	6.6
2	0.2	16.2	9.1	34.0	19.5	9.6	11.4
3	0.3	19.2	9.7	49.4	16.9	3.1	0.4
4	Woodsplinters	0.8	0.8	52.8	38.2	4.0	2.1
5	Few grains	0.6	0.6	63.0	28.8	3.8	2.4
6	None	0.6	0.6	46.9	43.6	4.8	2.5
7	None	1.0	1.3	55.4	30.6	5.5	5.5
8	None	1.0	1.1	49.0	38.8	4.5	4.8
9	None	1.3	1.0	39.2	44.6	7.2	6.3
10	None	0.6	0.4	48.8	46.0	3.0	0.5

cube salts are considerably heavier than water per unit of volume. Thus, the flake salts are lighter and more bulky than the cube salts. When packing the usual standard weight of 280 pounds of salt into the commercial package, the barrel for the cube salt need be only two-thirds as large as that for the flake salt.

*Size of salt grains.* Fifty-gram samples of salt were placed in the topmost of a series of sieves, of 20, 30, 40, 60, 80, and 100 mesh. The sieves were then shaken for five minutes, or until less than 0.3 gram additional salt passed through any screen in one minute. The percentages of salt passing through each sieve were determined, and are listed in table 1.



The above figures indicate that the great majority of all the salts passed through 40 and 60 mesh screens. The flake salts, however, proved considerably coarser than the cube salts and their range in crystal size was wider, while the cube salts show great uniformity in size of crystal.

*Microscopic examination of salts.* The salt grains were also studied under the microscope, using a magnification of 75 $\times$ , with

TABLE 2  
*Size and shape of crystals*

SALT NUMBER	TYPES OF CRYSTALS	SIZE OF INDIVIDUAL CRYSTALS	MESH OF CRYSTALS (ESTIMATED)
		mm.	
1	Flakes	0.16-1.6	50-150
2	Flakes	0.1-0.6	25-200
3	Flakes	0.15-0.90	25-150
4	Cubes	0.48	40-60
5	Cubes	0.13-0.24	40-100
6	Cubes	0.1-0.4	50
7	Cubes	0.16-0.43	40-100
8	Cubes	0.15-0.30	40-150
9	Cubes	0.08-0.32	50-100
10	Cubes	0.20	60

TABLE 3  
*Turbidity and color of saturated water solution*

SALT NUMBER	TURBIDITY	COLOR OF SOLUTION	SALT NUMBER	TURBIDITY	COLOR OF SOLUTION
1	108	Gray, turbid	6	40	Deep brown, turbid
2	5	Colorless, clear	7	8	Slight brown, clear
3	5	Colorless, clear	8	12	Slight brown, turbid
4	60	Brown, turbid	9	10	Slight brown, slightly turbid
5	3	Colorless, clear	10	5	Colorless, clear

the salts immersed in an oil. By applying slight pressure to one edge of the cover glass the crystals could be made to rotate in the oil thus permitting examination of all sides of the crystals. Measurements of the crystals were made by means of an eye-piece micrometer. Table 2 gives the predominating size and mesh, as estimated from these measurements.

*Turbidity and color of salt solution.* The turbidity was determined by comparing saturated solutions of the salt with turbidity standards prepared by mixing one gram of 200 mesh Fuller's earth in one liter of distilled water (A. P. H. A. Standard Methods of Water Analysis, 1925). These observations were made in 12-ounce bottles and also by the use of Nessler tubes.

Table 3 shows a considerable variation in the turbidity and color of the brines prepared from these salts. This character of the salt is not apparent where the use of the salt is confined to incorporation in butter, but when used in solution for soaking parchment wrappers and liners this turbidity becomes an objectionable property,—in fact, the turbidity of the brine from

TABLE 4  
*Sediment and insoluble matter*

SALT NUM- BER	APPEARANCE OF SEDIMENT	AMOUNT OF INSOLU- BLE MATTER	SALT NUM- BER	APPEARANCE OF SEDIMENT	AMOUNT OF INSOLU- BLE MATTER
		<i>per cent</i>			<i>per cent</i>
1	Gray brown	0.031	6	Dark brown	0.024
2	Light brown	0.007	7	Gray	0.009
3	Gray	0.004	8	Very dark brown	0.011
4	Dark brown and specked	0.032	9	Light brown	0.006
5	Light gray	0.003	10	Black	0.005

salts no. 1, 4, and 6, was so marked as to render these salts unfit for this purpose.

*Insoluble matter.* Saturated solutions of the various salts were passed through previously dried and weighed filter papers. These were again dried and weighed, giving the weight of insoluble matter in the dry salt. The appearance of the sediment and the per cent of insoluble matter are given in table 4.

The sediment was treated with 2 cc. concentrated hydrochloric acid. The solutions in all cases became perfectly clear. This suggests that the sediment was mainly salts of calcium and iron, probably largely calcium carbonate.

*Moisture content.* The moisture content of the salt has a direct bearing on its flowing property and caking tendency.

Ten gram samples of salt were dried at 115°C. until constant in weight. The results are shown in table 5.

*Caking tendency.* A small portion of each salt was left exposed to the air in a Petri dish for several days. All salts excepting nos. 7 and 9 showed considerable caking. These two salts still possessed their free-flowing property after such exposure to air. By referring to table 5, it will be noted that these two salts contained the least amount of moisture.

It was noted also that the addition to the dry salt of as little as 0.05 per cent water caused the salt to lose its free-flowing property and to appear damp. The addition of from 0.1 to 0.5 per cent water produced an even greater change in the same direction. When these moistened salts were exposed to the air they caked

TABLE 5  
*Moisture content of salts*

SALT NUMBER	MOISTURE	SALT NUMBER	MOISTURE
	<i>per cent</i>		<i>per cent</i>
1	0.08-0.10	6	0.12 -0.12
2	0.10-0.10	7	0.005-0.01
3	0.15-0.13	8	0.14-0.14
4	0.07-0.07	9	0.01-0.01
5	0.14-0.13	10	0.03-0.03

very profusely. This suggests that freedom from caking requires as nearly complete absence from moisture as possible.

The addition of 2 per cent calcium sulphate or 3 per cent potassium chloride to pure salt did not increase the caking tendency. The addition of 1 per cent calcium chloride or 1 per cent magnesium chloride, because of their deliquescent properties, however, caused the salt to cake. In the case of magnesium chloride the salt became and remained damp, but it did not cake to the same extent as with calcium chloride. These results suggest that the presence, in abnormal amounts, of calcium or magnesium chloride in butter salt is objectionable because these impurities cause the salt to become damp, or to cake, or both.

*Rate of solution.* Solubility tests of each salt were made as follows: A definite amount of salt was added to a given amount

of water. The liquid was kept in constant agitation by means of a motor stirrer and maintained at a constant temperature during the entire test. Samples were removed at carefully timed intervals and the percentage of salt determined by gravimetric analysis.

Since sodium chloride goes into solution very rapidly, it was necessary to remove samples quickly and at short intervals. This was accomplished by the use of 25 cc. pipettes cut off at the lower part of the bulb. Over this opening were stretched two layers of fine-mesh silk with a cotton pad or sediment disc between them.

TABLE 6  
*Solubility rates of butter salts*  
Percentage of salt in solution at various time intervals

SECONDS	SALT NO. 1	SALT NO. 2	SALT NO. 3	SALT NO. 4	SALT NO. 5	SALT NO. 6	SALT NO. 7	SALT NO. 8	SALT NO. 9	SALT NO. 10
5	20.20	22.40	21.28	20.82	19.75	21.03	20.54	20.04	21.14	20.82
10	23.72	24.33	23.63	23.66	22.95	23.76	23.27	23.38	23.84	23.67
15	24.67	25.22	24.80	24.72	24.32	24.99	24.63	24.59	24.87	24.74
20	25.35	25.60	25.24	25.30	24.94	25.54	25.13	25.22	25.39	25.27
25	25.62	25.87	25.45	25.73	25.45	25.91	25.65	25.68	25.70	25.72
30	25.75	25.97	25.67	25.71	25.52	26.07	25.78	25.94	25.92	25.90
40	26.10	26.19	25.96	25.86	25.98	26.34	26.12	26.22	26.09	26.14
60	26.25	26.32	26.19	26.17	26.26	26.35	26.24	26.43	26.24	26.31
90	26.33	26.39	26.29	26.31	26.22	26.51	26.30	26.47	26.30	26.38
120	26.35	26.43	26.34	26.40	26.48	26.37	26.39	26.54	26.32	26.40
180	26.38	26.43	26.36	26.44	26.47	26.51	26.40	26.55	26.34	26.40
240	26.30	26.42	26.37	26.44	26.54	26.66	26.39	26.56	26.33	26.39

The container was immersed in a water thermostat at 55°F. and each salt was properly tempered before addition to the water. The solubility determinations were made by placing two liters of water adjusted to exactly 55°F. in the container. The salt (740 grams) was weighed into a scoop so constructed as to discharge the entire amount of salt into the 2 liters of water in one second of time or less. With the water in vigorous agitation the salt was added and samples amounting to approximately 20 cc. were removed at definite intervals. In order to standardize this part of the procedure, the time for taking each sample was limited to exactly three seconds. The pipette was emptied by

inverting it so as to prevent crystals adhering to the outside of the silk from being washed into the sample bottle. The per cent salt was determined immediately by evaporation of 10 gram portions. To prove that no crystals of salt passed through the above filter pipette, check determinations were made with a Mandler diatomaceous filter of the Berkefeld type. The results of these tests of solubility rates are given in table 6. These results represent averages of several trials with each brand of salt.

Table 6 indicates that the difference in rate of solution between the several salts is very slight and not sufficient to justify any preference for one brand of salt over another.

Solubility tests were also made by using glass tubes 43 inches in length and  $\frac{1}{4}$  inch in internal diameter. The tubes were filled with water and 10 grams of the salt were discharged into each tube, allowing the salt crystals to settle through the water. A whitish turbidity was noted in the case of every salt but this disappeared quickly. This turbidity was due to air bubbles, either locked up in the salt or mechanically carried with the salt into the liquid. There was not much difference in the depth to which the salt crystals dropped before they dissolved, though there appeared to be a tendency for the flake salts (1, 2, and 3) to drop to a lower depth, a few crystals actually reaching the bottom of the tube.

This test cannot be considered a very dependable index of the rate of solution of the salt when used in butter. The concentration of the solution is not sufficient to match the concentration of the brine in butter and the fact that, as the crystals sink through the water they strike fresh water continuously, presents a condition entirely different from that existing in the salting of butter.

#### CHEMICAL ANALYSIS

. *Reaction of saturated brine to litmus paper.* A saturated solution of the salt was prepared with boiled, distilled water. Each solution was tested with litmus paper with the following results:

Neutral: Salts 2, 3, 5, 6, 8, and 10

Alkaline: Salts 1, 4, 7, and 9

*Methods of chemical analysis.* Samples for chemical analysis were dried on Petri dishes in the electric oven at 115°C.

*Phosphates.* One hundred grams of the salt were dissolved in 300 cc. of water. Ten cubic centimeters of nitric acid were added and then concentrated ammonia until almost neutral to litmus paper. Next, 40 cc. of 4 per cent ammonium molybdate were added. The mixture was heated to about 60°C. and allowed to stand at room temperature for one hour. Absence of bright yellow color or yellow precipitate indicated absence of phosphates.

*Iron oxide and alumina.* One hundred grams of the salt were dissolved in distilled water to saturation and about 10 drops of concentrated nitric acid added. The solution was boiled for about one-half hour to oxidize the iron, then a slight excess (0.5 cc.) of ammonia was added, boiling was continued until the odor of ammonia disappeared. The solution was allowed to stand overnight, then filtered, washed with hot water until free of chlorides, dried, and ignited in a small platinum crucible. The results are reported as  $\text{Fe}_2\text{O}_3 + \text{Al}_2\text{O}_3$ .

For the following analyses 100 grams of the dried salt were dissolved in distilled water and made up to 500 cc. One hundred cubic centimeters of this solution represents 20 grams of salt.

*Total lime.* One hundred cubic centimeters of the above salt solution (representing 20 grams of salt) were freed of iron and filtered. The solution was then heated to boiling. Ten cubic centimeters of 10 per cent oxalic acid were added. A few drops of methyl orange were added and the solution was neutralized with concentrated ammonia, added very slowly. One cubic centimeter excess of ammonia was added and the solution allowed to stand for three hours in a warm place. It was then filtered and washed once with 10 cc. of 1 per cent ammonium oxalate. The filtrate and washings were saved for the magnesium determination. The precipitate on the paper and in the beaker was dissolved with hot dilute HCl, then diluted to 100 cc. and again precipitated as before; it was filtered after three hours standing and washed with 1 per cent ammonium oxalate until free of chlorides. The filter was then ignited in a platinum crucible and the precipitate weighed as  $\text{CaO}$ .

*Magnesium oxide.* The washings and filtrate from the calcium determination were concentrated to 150 cc., 3 grams of diammonium phosphate and a few cubic centimeters of dilute HCl being added to clear the solution. The solution was allowed to cool and was then made alkaline with ammonia; 2 cc. excess  $\text{NH}_4\text{OH}$  were added. The mixture was allowed to stand overnight. It was then filtered, washed with a little dilute (1:10) ammonia, dissolved in HCl (1:1) and precipitated again and filtered as above. It was washed with ammonia (1:10) until free of chlorides, the test drop being neutralized with dilute  $\text{HNO}_3$  prior to addition of  $\text{AgNO}_3$ . The filter was ignited in a platinum crucible, weighed as magnesium pyrophosphate and reported as magnesium oxide.

*Sulphur trioxide.* To 100 cc. of the original salt solution (representing 20 grams of salt) 5 cc. of dilute HCl were added and the solution heated to boiling. Five cubic centimeters of a 10 per cent  $\text{BaCl}_2$  solution diluted to 100 cc. were then added gradually while hot, allowing to stand overnight. It was then heated and filtered while hot and washed with hot water until free of chlorides. The filter was ignited in a platinum crucible and weighed as  $\text{BaSO}_4$ , from which the  $\text{SO}_3$  content was calculated.

*Barium salts.* No barium salts can be present in these salts since soluble sulphates were present in all.

*Sodium chloride by gravimetric determination of chlorides.* Fifty cubic centimeters (10 grams salt) of the original solution were diluted to 500 cc. Twenty-five cubic centimeters of this solution, representing 0.5 gram of NaCl, were pipetted out and diluted to about 250 cc. in a 400 cc. beaker and 5 cc. of dilute nitric acid added. Twenty cubic centimeters of 10 per cent  $\text{AgNO}_3$  were then added slowly while stirring vigorously. It was allowed to stand in a dark place overnight, and then filtered on a Gooch crucible containing a layer approximately 0.5 cm. thick of long-fibre, acid washed asbestos, thoroughly washed with water prior to each use. The silver chloride precipitate was then washed with water containing a few drops of a solution of silver nitrate until only a faint test for nitrates was noted. Then it was finally washed with about 10 cc. of boiling hot water, and

TABLE 7  
*Chemical analyses of butter salts*  
 Percentage composition

SALT NUMBER	SODIUM CHLORIDE		CAL- CIUM SUL- PHATE	CAL- CIUM CARBON- ATE	CAL- CIUM CHLO- RIDE	MAGNE- SIUM SUL- PHATE	MAGNE- SIUM CARBON- ATE	MAGNE- SIUM CHLO- RIDE	SODIUM SUL- PHATE	FeO <sub>3</sub> + Al <sub>2</sub> O <sub>3</sub>	INSOLU- BLE MATTER	PHOS- PHATES	BARIUM SALTS	MOIS- TURE
	By direct analysis	By dif- ference												
1	99.19	99.76	0.073			1.38				0.002	0.031	0.00	0.00	0.06
2	98.92	99.58	0.302		0.078			0.025		0.002	0.008	0.00	0.00	0.105
3	99.48	99.88	0.027		0.048			0.041		0.003	0.004	0.00	0.00	0.140
4	99.11	99.19	0.699	0.046			0.027			0.008	0.031	0.00	0.00	0.070
5	99.08	99.18	0.756		0.059			0.006		0.001	0.003	0.00	0.00	0.135
6	98.34	98.59	1.225		0.116			0.037		0.006	0.027	0.00	0.00	0.125
7	99.69	99.90	0.010			0.010			0.071	0.003	0.009	0.00	0.00	0.005
8	98.67	98.90	0.996		0.060			0.026		0.002	0.012	0.00	0.00	0.140
9	99.57	99.94	0.038	0.007			0.011			0.001	0.006	0.00	0.00	0.010
10	99.39	99.77	0.197		0.022			0.013		0.003	0.005	0.00	0.00	0.032

Salts 3 and 9 were analyzed for KCl and found to contain less than 0.06 and 0.02 per cent respectively.



dried at a temperature of 105°C. overnight. The residue was placed in a large platinum crucible and heated gently with a Bunsen flame until the edges of the AgCl just began to fuse. It was then cooled and weighed as AgCl. From the figure thus obtained must be subtracted the silver chloride originating from chlorides other than that of sodium. Sodium chloride is so reported in the analyses.

*Tabulated results of chemical analyses of dairy salts.* The results listed in table 7 are on a moisture-free basis. In each case the method used was previously checked against mixtures of known

TABLE 8  
*Bacteriological analyses of salts*

SALT NUMBER	BACTERIAL COLONIES		MOLD COLONIES		TOTAL COLONIES	
	4 gram total	Per gram salt	4 gram total	Per gram salt	4 gram total	Per gram salt
1	6	1-2	0	0	6	1-2
2	8	2	1	<1	9	2-3
3	3	<1	0	0	3	<1
4	23	5-6	2	<1	25	6-7
5	4	1	4*	1-2	9	2-3
6	5	1-2	0	0	5	1-2
7	0	0	2	<1	2	<1
8	6	1-2	1	<1	7	1-2
9	3	<1	1	<1	4	1
10	9	2-3	4	1	13	3-4
Average.....	6-7	1-2	1-2	<1	8-9	2-3

\* 1 yeast.

chemical composition. Sodium chloride is reported both by direct analysis and by difference, i.e., by subtracting impurities from 100.

#### BACTERIOLOGICAL ANALYSIS

*Methods.* The various salts were examined for total number of bacteria and molds. This was done by plating on beef infusion agar. One gram of salt was weighed directly into a sterile Petri dish. Five cubic centimeters of sterile distilled water were added to dissolve the salt before pouring the agar. Incubation was at

21°C. for four days, followed by two days at 37°C. Two examinations of each salt were made from different parts of the barrel.

Preliminary plating was also done on whey agar with 1 cc. of a 1 per cent solution of lactic acid per plate to give favorable conditions for mold growth. It was found, however, that beef infusion agar gave fully as positive results and consequently whey agar counts were discontinued.

A 10 per cent solution of each salt was also made in sterile distilled water and 1 cc. of this solution was added to the Petri dish in order to allow a large dilution and to avoid any inhibitory action which might be exerted in the case of the greater salt concentration. This method, however, gave no colonies at all on some plates and only one colony on others, indicating that the plating of one gram of salt in each dish without dilution was the only method to be relied upon for securing the true germ content of the salt. The results are given in table 8.

The results in table 8 indicate that all of the salts studied are low in germ life and none of them, if handled properly and kept in the condition in which they exist in their original container, the sealed barrel, would be a source of contamination of any consequence in the manufacture of butter.

#### EFFECT OF SALT ON FLAVOR, BODY, AND COLOR OF BUTTER

*Method.* A sufficient quantity of unsalted butter taken from a factory churning was used for each entire salt series. Thirty pounds of this butter were worked up with each salt in a small combined churn and worker. The butter was first worked in water in an effort to have all lots of butter in the same condition as to body, texture, and temperature before and at the time the salt was added. The amount and temperature of the water used and the number of revolutions worked were the same for all lots.

After draining the water out of the churn the same amount of salt was added to the butter in each case (aiming at 3 per cent salt in the finished butter) and the same amount of water was used to wet the salt in the trench. After closing the trench the butter was worked the same length of time for each salt. It was

then packed in 30 pound tubes and placed in storage at 40°F. until scored. The churn was washed out between consecutive lots of butter to remove all remnants of brine. Two entire series of this experiment were made. The tubs were all numbered for scoring and no judge had any knowledge of the identity of any of the tubs. The butter was examined by three judges for flavor, body, color, and salt. Particular attention was paid to the detection of bitterness, brininess, and the presence of undissolved salt as "grit."

*Results.* Careful examination and re-examination of each tub of butter by the three judges failed to reveal any consistent or significant differences. Comparisons of the various lots of butter for bitter and briny flavors gave convincing evidence that the brand of salt had no effect. They further showed very clearly that it is the method employed in the working of the butter and in the incorporation of the salt that is of primary importance and that controls the presence or absence of the objectionable briny, bitter flavor in butter. No difference in the shade or uniformity of color was detected among the several churnings.

The presence of "grit" due to undissolved salt was not restricted to any particular brands or types of salts, and consistent results were not always secured with the same salt. This finding has been confirmed under practical conditions in plants actually having difficulties in the complete solution and incorporation of salt in their butter. A change in the type of salt crystals from flakes to cubes produced no appreciable difference in the degree of grittiness and when the conditions of incorporating the salt and working the butter were changed, the grittiness disappeared with both types of salts.

Comparisons between a number of the salts were also made on a large scale under commercial operation in the Chicago factory. Separate churnings from the same cream out of the same vat were worked into salted butter, a different salt being used for each churning. Thus, here too, all interfering factors influencing the flavor were eliminated, facilitating detection of flavor differences due to salt. Careful examination of this commercial butter failed completely to show any differences in flavor, body, texture, and color.

On the basis of these findings we are forced to conclude that, as far as the brands of salts included in this experiment are concerned, the particular brand had no effect on the quality of the butter.

#### EFFECT OF IMPURITIES IN SALT ON BUTTER

*Method.* In this experiment such chemical impurities as are most likely to be encountered in salt were mixed with different portions of a pure salt before adding the salt to the butter. The impurities used were potassium chloride, calcium chloride, magnesium chloride, calcium sulphate. Potassium chloride possesses a penetrating, pungent character, and magnesium and calcium chlorides are bitter and biting to the tongue. It would be expected that their presence in butter salt in abnormal amounts would tend to impart a bitter flavor to the butter. Calcium and magnesium chlorides are further objectionable because of their deliquescent property. Their presence causes salt to absorb moisture from the air and to cake (see Physical Analysis). The amount of impurity mixed with the salt was greatly in excess of that present in any of the ten salts used in this investigation. Only one impurity was added to a salt for a single test. Each set of tests also contained a check churning of the same butter in the manufacture of which the pure salt only was used. The mixed impure salt contained the following percentages of impurities respectively:

	<i>per cent</i>
Potassium chloride . . . . .	3
Calcium chloride . . . . .	1
Magnesium chloride . . . . .	1
Calcium sulphate . . . . .	2

The salts prepared with the above impurities and the pure salt with no added impurity were worked into different lots of butter from the same churning. In each case the butter was so salted and worked as to secure a final moisture and salt content of as nearly 16 and 3 per cent respectively as possible. Four sets of comparisons were made, each consisting of one pure and two impure salts. Three judges examined each set of samples. The

judges were ignorant of the salt which the different samples represented.

*Results.* In spite of repeated scoring no consistent differences in flavor between the various tubs of butter could be established. These results substantiate and explain the absence of any visible difference in the flavor of the butter salted with the different brands of salt as discussed under "Effect of salt on flavor, body, and color of the butter." If the addition of these comparatively large percentages of impurities to the salt fails to have a sufficient influence on the quality of the butter to be noticeable to taste, it may be readily understood why different brands of salt in which the several chemical impurities are present in much smaller percentages fail to produce any differences in the quality of butter detectable by the sense of taste.

These findings suggest that the importance of slight differences in the chemical composition of butter salts has been somewhat exaggerated. It should be understood, however, that in these experiments the moisture was well incorporated and there was no indication of leakiness. In the case of very leaky butter, the free brine would undoubtedly give the palate a better opportunity to detect the bitterness which accompanies these impurities. Because of this possibility we must conclude that the use of butter salts containing excessive amounts of chemical impurities may jeopardize the flavor of the butter and is, therefore, to be avoided.

#### SUITABILITY OF SALTS FOR BRINE TREATMENT OF WRAPPERS AND LINERS

In the preparation of the butter package it is common practice to soak the parchment wrappers and liners in saturated brine for the purpose of preventing or retarding mold growth on the surface of the butter. In order to have this brine sterile it is usually heated to the boiling point by injecting live steam. In the case of some salts this heating of the brine causes the formation of a heavy precipitate, often of marked coloration, and the collection of a sediment, all of which is objectionable.

Tests made with the different salts gave varying results. The color, turbidity, and amount of sediment in the brine are given in table 9.

TABLE 9  
*Comparison of brines for treatment of parchment*

SALT NUMBER	APPEARANCE OF BRINE	TURBIDITY OF BRINE	SEDIMENT IN 1 QUART OF BRINE
			<i>mgm.</i>
5	Good	5	10.4
2	Good	6	4.8
3	Good	7	3.0
7	Fair	15	10.2
10	Fair	11	4.5
4	Poor	80	53.5
1	Poor	160	
6	Poor	60	44.3
8	Poor	30	12.3
9	Poor	20	11.7

The first three salts listed in the above table gave a relatively clear brine, and salt no. 3 was judged best. The salts marked "poor" produced brines which could not be used for soaking parchment wrappers. Brines no. 1, 4, 6, and 8, were very turbid and brown in color. Salt no. 9 produced brine of intense yellowish brown color with a large amount of brown sediment. It cleared somewhat, however, upon standing overnight and at that time it was not as turbid as numbers 6 and 8. It is obvious that only such salts should be used for brine treatment of parchments as will produce a brine that does not place any deposit or sediment on the wrapper which lies next to the butter.

#### SUMMARY

1. Ten leading butter salts, representing three flake salts and seven cube salts, were studied with reference to their physical, chemical, and bacterial properties as related to their effect on flavor, body, texture, and color of butter.

2. Some of the salts were badly caked in the barrel while others were free-flowing. The most freely flowing salts were those that showed the greatest freedom from chemical impurities and that

were lowest in moisture content. Numerous tests showed that the drier the salt the less its tendency to lump. As the moisture content of the salt increases the salts lose their free-flowing property and cake profusely. For similar reasons the presence in salt of calcium chloride and magnesium chloride, which are highly deliquescent, diminishes the free-flowing property of the salt and causes it to lump.

3. The flake salts were found to be considerably more bulky than the cube salts. Taking the weight of a given volume of water as one, the weight of the flake salts averaged 0.86 and of the cube salts 1.26. With the weight of the commercial package of salt standardized to 280 pounds per barrel, the barrel for cube salt need be only two-thirds as large as the barrel for flake salt.

4. Several of the salts contained foreign matter in sufficient amounts to produce a very turbid brine and dirty color when dissolved in water. Such salts are objectionable, particularly when intended for use in the preparation of brine for the treatment of parchment liners, circles, and wrappers.

5. The salts in the original containers were found to be bacteriologically clean. The bacterial counts ranged from less than one colony per gram of salt to seven colonies, averaging from 2 to 3 colonies for all salts. Unless contaminated in the creamery after the barrel is opened, due to improper storing or handling, these salts may be considered entirely negative as a possible source of bacterial contamination of butter.

6. The great majority of the crystals in each salt passed through 40 and 60 mesh screens. The flake salt showed a considerably coarser grain and a wider range of crystal size than the cube salt. Thus, of the flake salts about 60 per cent of the crystals passed through 40 and 60 mesh screens while 27 per cent required a coarser screen. Of the cube salts, approximately 90 per cent of the crystals passed through the 40 and 60 mesh screens while only 1.7 per cent required a coarser screen.

7. The ten salts were very similar in their rates of solution. During the first twenty seconds there was a tendency for the flake salts to show slightly greater rapidity of solution than the

cube salts but at the end of twenty-five seconds the amount of salt dissolved averaged the same for both types of crystals. At the end of two minutes the solution of each salt was complete. After the first five seconds each liquid contained 19.75 per cent or more of salt in solution. This salt concentration is fully equal to the strength of the brine in butter containing 3 per cent salt. This rapidity of solution of each of the ten salts suggests that such minute differences in solubility rate as were observed between individual brands and between the two types of salt are too slight to be of any significance from the standpoint of preference for any one salt in butter manufacture.

8. The percentage of sodium chloride in the 10 salts, as determined by direct analysis, ranged from 98.34 to 99.69 per cent, averaging 99.14 per cent. The sodium chloride content as determined by difference ranged from 98.59 to 99.94 per cent. Phosphates and barium salts were entirely absent in all salts. The largest chemical impurity consisted of calcium sulphate ranging from 0.01 to 1.225 per cent. Small amounts of calcium and magnesium chlorides, magnesium and sodium sulphates, and calcium and magnesium carbonates, and traces of iron, were also present. The insoluble matter ranged from 0.003 to 0.031 per cent and the moisture from 0.005 to 0.14 per cent.

9. When working these salts into butter, both under experimental conditions and in commercial manufacture, no differences in flavor, body, texture, and color could be detected in the finished butter. Likewise, the addition to the pure salt of impurities in relatively large amounts, such as 2 per cent  $\text{CaSO}_4$ , 3 per cent  $\text{KCl}$ , 1 per cent  $\text{CaCl}_2$ , and 1 per cent  $\text{MgCl}_2$ , failed to have any noticeable effect on the flavor of the butter.

10. These findings suggest that, while chemical purity in butter salts is highly desirable, such small amounts of chemical impurities as are found in the above standard butter salts are incapable of impairing or changing the quality of butter.



## DESTRUCTION OF BOTULINUM TOXIN BY MILK BACTERIA\*

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Data have been published elsewhere, (Sherman, Stark, and Stark, (8)) which show a definite, though slight, destruction of botulinum toxin by intestinal bacteria. The intestinal bacteria studied are also commonly found in milk. The present paper reports work of a similar nature dealing with other types of milk bacteria in pure culture together with some observations on the action of a mixed flora.

The unique freedom of milk and milk products as agents in the dissemination of botulism is of great interest. Although fresh milk may ordinarily be used before bacterial growth has been sufficiently extensive to be dangerous from the standpoint of botulinum poisoning, such is not at all the case with cheese and certain other milk products. Indeed, from the standpoint of chemical composition, cheese would appear to be an excellent medium for the production of this toxin. The explanation which might appear valid is that the acidity of most types of cheese is sufficiently high to inhibit the growth of *Clostridium botulinum* but such an explanation would not hold for all types of cheese, and from some of the experimental results which have been published concerning the acid limits for growth of this organism, would indicate that a complete explanation cannot be founded entirely on the basis of acidity in the case of any type of cheese.

Jordan (5) puts the acid limit of growth of *Clostridium botulinum* at pH 4.9. It is well known that milk develops a lower pH than this after it is coagulated by acid in normal souring. This, however, would not account for the disposition of the toxin which might be produced while *Clostridium botulinum* is growing, before sufficient acid is produced to inhibit its

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action. Bronfenbrenner and Schlesinger (1) found that increasing the acidity to pH 4.0 caused botulinum toxin to become many times more toxic. Dozier (3) says, "The decline in numbers of viable organisms is followed by autolysis, which is probably the mechanism of toxin formation."

Nevin (7) records an instance in which three persons died of botulinum poisoning from eating home-made cottage cheese. She was able to isolate a type B strain of *Clostridium botulinum* from a sample of this cheese. In 1914, when these deaths occurred, but little information was available concerning this type of poisoning and "home-made cottage cheese" tells us little of the nature of the product from which the sample came.

Edmondson, Thom, and Giltner (4) inoculated three kinds of milk, designated by them as "sterilized," "laboratory pasteurized" and "commercial" (raw), with toxin-free spores of *Clostridium botulinum* and incubated them at various temperatures. Guinea pigs were fed this milk in order to test for the presence of toxin. They found that toxin was produced in the sterilized and laboratory pasteurized but not in the raw commercial milk. They say, "*B. botulinus* failed to produce toxin at any temperature in the low grade commercial product. No analysis of this result has been possible."

With slight changes, the experiment of Edmondson, Thom, and Giltner has been repeated by us. Only two incubation temperatures, 20° and 37°C. were employed. The samples of milk were inoculated into guinea pigs subcutaneously. Koser, Edmondson, and Giltner (6) found at least 500 times as much toxic material required when given by mouth to produce a similar effect as when injected intraperitoneally. In the first experiment, the results of which are recorded in table 1, the milks were lightly inoculated with detoxified spores of *Clostridium botulinum* and incubated for twenty-one days. At the end of this period guinea pigs were inoculated with 0.5 cc. amounts. In those samples in which noticeable proteolysis was absent filtration was difficult and in such cases the inocula were not filtered. Enough checks were run with the filtered and unfiltered product to convince us that the guinea pigs inoculated with unfiltered milk cultures, which

did not show proteolysis, did not die because of secondary infections caused by other bacteria which were present in the milk. The milk used in these experiments was of ordinary market quality.

All of the pigs in this experiment died from one to ten days after being inoculated, except one pig inoculated with raw milk which had been incubated at 20°C.

TABLE 1

*The effect of bacteria on the accumulation of toxin in milk inoculated lightly with detoxified spores of Clostridium botulinum*

VARIETY OF MILK	INCUBATION TEMPERATURE	TOXICITY TO GUINEA PIGS
	°C.	
Sterilized.....	37	Dead in 24 hours
Pasteurized.....	37	Dead in 8 days
Untreated.....	37	Dead in 2 days
Sterilized.....	20	Dead in 24 hours
Pasteurized.....	20	Dead in 10 days
Untreated.....	20	Survived

TABLE 2

*The effect of bacteria on the accumulation of toxin in milk inoculated heavily with detoxified spores of Clostridium botulinum*

VARIETY OF MILK	INCUBATION TEMPERATURE	TOXICITY TO GUINEA PIGS
	°C.	
Sterilized.....	37	Dead in 24 hours
Pasteurized.....	37	Dead in 2 days
Untreated.....	37	Dead in 10 days
Sterilized.....	20	Dead in 2 days
Pasteurized.....	20	Dead in 9 days
Untreated.....	20	Dead in 9 days

In repeating this experiment, the results of which are recorded in table 2, heavier inoculations were used, approximately 50,000 detoxified spores per cubic centimeter of milk. The incubation period was fifteen days. The amount injected into the test pigs was the same (0.5 cc.).

All of the guinea pigs given any one of the three kinds of milk,

incubated at either 20° or 37°C., were dead at the end of ten days. A possible interpretation of these results will be given later.

In order that we might have more definite information, the action of certain types of milk bacteria upon botulinum toxin was tested. Twenty-seven cubic centimeters of sterile meat infusion nutrient broth were placed into sterile test tubes. To each of these tubes of broth were added 3 cc. of botulinum toxin fluid. This toxic material was prepared by inoculating *Clostridium botulinum* into sterile milk and incubating under anaerobic conditions for seven days at 37°C. At the end of this incubation period the peptonized milk was filtered through a

TABLE 3  
*Action of milk bacteria upon botulinum toxin*

	M.L.D. TOXIN INJECTED			
	1	2	4	8
Controls.. .. .	+	+	+	+
<i>Streptococcus lactis</i> .....	-	-	-	+
<i>Lactobacillus casei</i> ....	-	-	-	+
<i>Proteus vulgaris</i> .....	-	-	+	+

+ = death of guinea pig with typical symptoms of botulism. - = survival of guinea pig.

Berkefeld filter to free it from organisms. This sterile toxic material was held for six months in a cold room having a temperature just above the freezing point. The toxicity of this material as determined just previous to its use in these experiments was approximately 100 M.L.D. per cubic centimeter. This would give us a toxin-broth mixture containing, at the beginning of the incubation period, approximately 10 M.L.D. per cubic centimeter. Table 3 shows the results of the action of certain milk bacteria upon botulinum toxin. In this table the unit amounts of toxin given are in terms of approximate M.L.D. values as determined on the control tubes after incubation.

After fifteen days' incubation at 37°C. and filtration, this toxin-broth mixture into which no organisms were placed was

found to contain about 1 M.L.D. of toxin per cubic centimeter. Dack (2) found a reduction of two times the original titer from ten days' incubation at 37°C. when using a toxin which had been stored for two months in the refrigerator.

The results from the action of these milk bacteria upon botulinum toxin show a very definite destruction of this toxin. Since it has been shown that certain prevalent milk bacteria have the power to destroy botulinum toxin, we are able to make some interpretations of the results obtained by Edmondson, Thom, and Giltner (4). Twenty degrees Centigrade is near the lower temperature limit for growth of *Clostridium botulinum*, while many of the bacteria common to milk grow well at this temperature. The organism of botulism is usually strongly proteolytic and its activity is hindered by the presence of high acidity. The toxin formed in milk, pasteurized or raw, is slowly destroyed by the milk types. When given by mouth instead of being injected, much more toxin is required to cause death.

In Edmondson, Thom, and Giltner's experiments we believe the small amount of toxin produced, the destruction of this toxin by the milk bacteria, and the larger quantity of toxin required to kill when given through the digestive tract explain the survival of the animals to which they fed "commercial" milk. The same line of reasoning will explain why the guinea pigs which we inoculated subcutaneously with raw milk, which had previously been inoculated with detoxified spores of *Clostridium botulinum*, died.

In the case of the deaths from botulism poisoning reported by Nevin (7) it is likely that the cottage cheese was heavily contaminated with the toxin producing organisms, and the persons dying probably ate large quantities of the cheese. Edmondson, Thom, and Giltner (4) were unable to produce poisonous cottage cheese by inoculating fresh, raw milk with *Streptococcus lactis* and "a few" detoxified botulinum spores, incubating the milk for two days at 20°C., and the cheese for four days at 16°C. after manufacture. The toxicity of this cheese was tested by feeding it to guinea pigs. These results are as would be expected in the light of our data.

It is believed that knowledge of the destructive action of intestinal and milk types of bacteria upon botulinum toxin will be of value to the canning and dairy industries of the country and the consuming public. The intestinal types with which we have worked are always present in market milk. We believe these results explain, in part, why milk and dairy products, which undoubtedly at times contain *Clostridium botulinum*, fail to cause poisoning.

#### SUMMARY

It has been shown that certain bacteria commonly found in milk have the power of destroying toxin produced by *Clostridium botulinum*. The data reported in this and a previous paper have shown that this destruction may be caused by *Streptococcus lactis*, *Lactobacillus casei*, *Bacterium coli*, *Bacterium communior*, *Bacterium aerogenes*, and *Proteus vulgaris*.

It is believed that these results explain in part the fact that milk and dairy products are seldom if ever agents in the dissemination of botulism.

The fact that *Streptococcus lactis* and *Lactobacillus casei* have the power to destroy botulinum toxin is of particular interest in connection with cheese, since in most types of cheese *Streptococcus lactis* is the predominating organism during the early stages of ripening, while *Lactobacillus casei* is one of the predominating organisms in all types of cheese during the later stages of ripening.

#### REFERENCES

- (1) BRONFENBRENNER, J., AND SCHLESINGER, M. J. 1922 Some of the factors contributing to toxicity of botulinus toxin by mouth. Jour. Amer. Med. Assoc., lxxviii, 1519-21.
- (2) DACK, GAIL M. 1926 Influence of some anaerobic species on toxin of Cl. botulinum with special reference to Cl. sporogenes Jour. Infect. Dis., xxxviii, 174-181.
- (3) DOZIER, CARRIE CASTLE 1924 Optimum and limiting hydrogen ion concentration for B. botulinus and quantitative estimation of its growth. Jour. Infect. Dis., xxxv, 105-133.
- (4) EDMONDSON, RUTH B., THOM, CHARLES, AND GILTNER, L. T. 1923 Experiments with Bacillus botulinus under household conditions. Amer. Food Jour., xviii, 143-145.

- (5) JORDAN, E. O. 1923 General Bacteriology. W. H. Saunders Co., Philadelphia. Page 407.
- (6) KOSER, S. A., EDMONDSON, R. B., AND GILTNER, L. T. 1921 Observations on *Bacillus botulinus* infection of canned spinach. Jour. Amer. Med. Assoc., lxxvii, 1250-53.
- (7) NEVIN, MARY 1921 Botulism from cheese. Jour. Infect. Dis., xxviii, 226-231.
- (8) SHERMAN, J. M., STARK, C. N., AND STARK, PAULINE 1927 The destruction of botulinum toxin by intestinal bacteria. Proc. Soc. Exper. Biol. and Med., xxiv, 546-547.

# BOVINE INFECTIOUS ABORTION

## INCREASED PRODUCTIVITY OF AN ABORTION-FREE DAIRY HERD\*

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### INTRODUCTION

The clean herd under discussion here is the regular Connecticut Agricultural College herd, and comprises at this writing 115 head, of which 50 are milch cows, and the remainder young stock and bulls. This herd is made up of four breeds, Jersey, Guernsey, Ayrshire and Holstein, all at present pure-bred.

The earlier investigations<sup>1</sup> (1) conducted over a period of several years convinced the writers that effective barriers against the spread of the Bang abortion disease cannot be erected by ordinary methods of sanitation and temporary isolation. Continued experience with the agglutination and complement fixation tests and confidence in their results led them to the conclusion, however, that eradication is thoroughly feasible. Owing largely to the loss of the College dairy barn in 1919 which was not adequately replaced until 1924, the complete elimination scheme was not put into execution in the College herd until the winter of 1924-1925.

Almost from the beginning of the series of investigations on infectious abortion it became apparent to the writers that calves are not permanently infected at birth, or later during the milk feeding period, even though they are born of positive dams; and, therefore, that calves constitute no serious problem until they approach sexual maturity.

In anticipation of the completion of the new maternity and young stock barns the practice was begun in 1923 of removing

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<sup>1</sup> This is the thirteenth paper of a series on the subject of bovine infectious abortion. See bibliography (1).



female calves at weaning time (about six months) to quarters apart from the regular herd. Even though the heifers were later bred to non-reacting herd bulls which were used on both reacting and non-reacting cows, no difficulty was experienced in keeping the heifers abortion-free.

During all of 1924 a rather severe culling of the milking herd was under way. This culling involved the removal of animals which calved prematurely, and abortion reacting cows of mediocre milking capacity. As a consequence of this treatment of the herd, and because only an occasional animal was added, very few new reactors appeared in the herd during the nine months preceding the adoption of the full eradication scheme.

The new barn, which was separated from the main milking barn by a connecting storage building, was completed and put to practical use in November, 1924. The heifers that had been reared in separate quarters, of which several were well advanced in gestation, were at this time placed in the new barn. Although the policy of removing all reacting animals from the milking herd was not put into effect until February and March of the following year, 1925, infection did not spread to the new (young stock) barn. While the new barn contained maternity stalls, only non-reacting cows were placed in it.

Two of the heifers which had been reared in separate quarters became reactors in 1925. This was accounted for by the transfer to the mature herd of a few heifers after calving, before the reactors had been removed from the milking barn. These two newly reacting animals were, of course, removed with the other reactors at the time complete segregation was begun.

Some of the reactors were removed in February and the balance in March 1925, fifteen in all. They were taken to an old barn about three quarters of a mile distant from the main barns. This reacting herd was retained to maintain the general milk supply which was admittedly somewhat below normal because of the rather aggressive policy of disposing of reactors in the preceding nine months. Furthermore, some of the best producing cows were among the segregated reactors and they were needed as breeders to supply high grade calves for building up the clean herd.

Following the removal of all reactors, only one cow has been lost from the supposedly clean herd by becoming a reactor. This animal gave a suspicious reaction in May and June, 1925, and in the July test proved to be a complete reactor in the sixth month of gestation. This heifer had suffered from an acute attack of scours when only a few days old which resulted in a slight physical deformity. She had come from a positive dam and had reacted continuously until she was at least a year old. This heifer was one of the rare exceptions to the observed rule (Bul. No. 93) that heifers, no matter what their origin, are negative to the blood tests after they are from five to six months old. She was removed as a reactor, and later (September) aborted.

There can be no doubt that the rapid elimination of, and the apparently complete freedom from infection of this herd over a period of more than three years are due to stabilization or delimitation of the infection just before and at the time the reactors were removed.

Monthly tests were conducted on the clean herd for more than a year after complete segregation and following this the herd has been subjected to quarterly tests. The uniformity with which the results were negative, except for an occasional non-specific doubtful reaction, is further evidence of the value of the serological methods. These results are especially significant when we consider the high production and calving record of the herd.

In less than a year after the removal of all reacting animals the gross milk yield of the abortion-free herd was back to normal, due to the rapid addition of young, freshening cows. Since then the production record has been consistently greater than at any other time in the history of the herd.

The reacting herd has been continued, with some sales and new additions, for further study. The bulls of the non-reacting herd have been used with the reacting cows, but never without thorough disinfection of the sheath. The calves from the reacting herd have been transferred when one or two days old to the young stock barn of the clean herd. As calves from positive dams may carry the Bang bacillus temporarily from uterine exposure and by having ingested infected milk, they were treated ex-

ternally with disinfectant solution and placed in a small isolation pen for 50 to 60 days before they were allowed contact with other animals. More recently we have endeavored to bring new calves of the positive herd to the clean calf barn before they nursed. Such calves, as is now well known, react and continue to react negative, though they are from positive dams, and long continued isolation is not necessary. No feeding troubles have been encountered as the result of this practice.

#### ECONOMIC LOSSES FROM THE BANG ABORTION DISEASE

In an earlier Station Bulletin (Bul. No. 135) it was shown that the abortion reacting cows in the College herd gave a return of \$44.01 less per cow per year over feed cost than the non-reacting animals. These figures were compiled from data covering an eleven year period in which reacting and non-reacting animals were in constant association with each other, and were based upon calf losses, milk yield, and depreciation in the value of animals from actual sales.

It should be understood that when the losses from premature calving (the calf itself) and from subnormal subsequent lactation were included the actual loss per cow for that period was much greater than \$44.01. This figure is based upon the average yearly performance for full time in milk, which was practically four years per cow. Since the life time abortion rate among reactors was found to be 26.2 per cent (Bul. No. 123), the average number of abortions per cow was only about one during the four years residence as a milch cow. All gestations of less than 265 days were considered abortions.

Thompson (2) found that his segregated reacting Guernsey herd produced milk at a cost of 16.8 cents per quart, against 10.4 cents per quart for the non-reacting herd.

Sims and Miller (3) reported the milk production for two years by a herd that had been tested and the reactors and non-reactors segregated. The negative herd averaged 7343 pounds of milk per cow in 1923, and 6291 in 1924, and the positive 4544 pounds in 1923 and 3262 pounds in 1924. In the presumably clean herd one abortion occurred in 78 freshenings, and in the infected herd

10 cows out of 42 aborted. No statement of costs is given, except that the reacting herd was abandoned at the end of the second year because of the heavy losses.

Quite recently Newsom and Cross (4) reported abortion segregation results on the dairy and beef herds of the Colorado Agricultural College. They stated that it took ten and one-half months to eliminate the disease from the beef herd in which 16 animals or 43 per cent of the original herd were removed as reactors. A loss of \$92.38 for each of the 16 reactors was estimated as the depreciation sacrifice. With the dairy herd eighteen and one-half months were required for complete eradication, with a sacrifice of 15 cows or 34 per cent of the original herd. The depreciation in this instance was estimated to be \$70.55 per reacting animal. The reactors in the two herds were slaughtered, which accounts for the high depreciation. The premature calvings previously had ranged from zero to 17 per cent in the beef herd, and from zero to 23 per cent in the dairy herd, during the years 1917 to 1926, inclusive. In both of these herds slow conception constituted a serious menace at times.

Barnes (5) states that where 10 per cent of the herd aborts the equivalent of the entire herd is lost every five years. In the Storrs herd, which had an average of 19.5 per cent abortions over a period of 21 years, the equivalent of the whole herd was lost every four years. Barnes states further that the loss from abortion in Pennsylvania amounts to \$5,000,000 annually.

#### RESULTS OF THREE YEARS' OBSERVATIONS ON THE ABORTION-FREE CONNECTICUT COLLEGE DAIRY HERD

It is the chief purpose of this paper to demonstrate the value of eradication of the Bang abortion disease, through increased financial returns.

Data will be presented under the following headings: (1) calving record; (2) live stock sales; (3) milk yield; and (4) other improvements.

#### *Calving records*

Calving data have been published from time to time in previous bulletins. These are summarized in table 1, together with the

more recent and unpublished figures for 1925, 1926 and 1927. Several distinctive and significant facts may be deduced from this table.

TABLE 1  
*Summary of calving conditions during the years 1904 to 1927, inclusive*

YEAR	NUMBER OF COWS	BREEDING COWS WHICH CALVED		CALVING COWS WHICH ABORTED		REACTING COWS	
		Number	Per cent	Number	Per cent	Number	Per cent
1904					46.1		
1905					25.9		
1906					19.3		
1907					24.0		
1908					11.5		
1909					15.1		
1910					17.6		
1911					27.9		
1912					20.0		
1913					20.0		
1914	37	28	75.7	7	25.0	21	56.8
1915	39	31	79.5	2	6.5	16	41.0
1916	44	35	88.4	4	10.5	14	31.8
1917	45	34	77.7	3	8.6	12	26.7
1918	39	31	89.7	2	5.7	8	20.5
1919	46	37	82.6	12	31.6	16	34.8
1920	44	32	72.7	12	36.4	20	45.5
1921	45	40	88.8	10	20.0	21	46.7
1922	43	38	88.3	6	15.8	17	39.5
1923	46	39	80.5	11	28.2	21	45.7
1924	49	39	80.0	6	15.4	24	49.3
Average 21 years.....	43.4	35.0	80.5	6.8	19.5	17.3	39.8
1925	39	35	87.4	1	2.9	1	2.5
1926	49	49	100.0	3	6.1	0	0.0
1927	49	45	91.8	0	0.0	0	0.0

1. From 1904, when the first complete records were available and at which time presumably the infection was introduced into the herd, the abortion rate rose and fell at practically eight year intervals between the high peaks, running as high as 46.1 per

cent, and never lower than 5.7 per cent, in any calendar year up to the time of eradication.

2. The average number of premature calvings (before the 265th gestation day) during a period of twenty-one years through 1924 was 19.5 per cent.

3. After the reacting cows were segregated in 1925 one new reactor appeared in the supposedly clean herd (as previously described); since then no animals in the clean herd, nor any of their descendants in the herd, have reacted.

4. Up to the end of 1927 there have been 129 calvings in the abortion-free herd. Four of these were premature: this gives a rate of 3.1 per cent, which is not far from that observed previously in over 500 calvings of non-reacting cows (Bul. No. 123). Two of these abortions were satisfactorily accounted for, but there is no explanation for the one that occurred with a first calf heifer and another that occurred with a second calf young cow. Three of these aborting cows are breeding regularly now; the fourth is dead.

5. The breeding efficiency of the herd, that is the percentage of milking cows that calve in each calendar year, rose to 100 per cent in 1926, and in 1927 it was 91.8 per cent. Both of these are high peaks in the history of the herd. In 1926 one cow did not calve during the calendar year, but another calved twice during the year. A cow is classed as a breeding cow in every calendar year that she is in the herd after her first calving.

6. For the first time in the herd's history not a single premature calving occurred in the herd in 1927.

Perhaps the reader will wonder how the *reacting* herd has fared during the last three years period. About half of the original group remains and a few reacting cows, purchased here and there, have been added. There have been 29 calvings, ten or 34.5 per cent of which have been premature.

#### *Live stock sales records*

Obviously when the abortion disease is rampant in a herd the surplus stock sales must suffer. Table 2 presents these facts for

the twelve-year period beginning in 1916 and admits of some striking deductions.

1. Following an abortion storm, which abated in 1915 (see table 1), the stock sales were still light in 1916, but rose rapidly with the four good breeding years 1915, 1916, 1917 and 1918, when the total sales reached 32 head, at a value of \$2,868.40 in 1919. During this time we were encouraged to believe that the disease would be stamped out of the herd, but in the latter part of 1919 the test showed unerringly that the disease again was spreading and that an

TABLE 2  
*Live stock sales from the College dairy herd*

YEAR	TOTAL ANIMALS SOLD	TOTAL VALUE OF SALES	NUMBER OF CALVES VEALED	NUMBER OF COWS SOLD		NUMBER BULLS AS BREEDERS
				For meat	As producers	
1916	11	\$653.00	1	4	1	5
1917	19	1,182.14	2	5	6	6
1918	13	1,532.50	1	3	7	2
1919	32	2,868.40	10	7	6	9
1920	22	1,995.00	3	12	3	4
1921	22	821.00	3	12	3	4
1922	23	781.00	8	9	5	1
1923	24	1,329.00	6	4	11	3
1924	24	1,059.32	9	5	5	5
1925	30	2,173.50	8	9	8	5
1926	33	1,113.00	14	7	4	8
1927	38	3,652.65	5	3	11	19
Total.....	291	\$19,160.51	70	80	70	71

abortion storm was inevitable. It came and many of the second and third calf cows that had thus far escaped, as well as a majority of the first gestation heifers, became reactors, of which a goodly proportion aborted.

2. The slump in sales from 1920 to 1922 is particularly notable; also a decided shift in the proportion of cows sold as producers to cows sold to the butcher.

3. In 1923, 1924 and 1925 the sales of cows as producers were considerably increased as compared with those which went for beef, due to the disposal of some grade animals that had been

purchased for experimental feeding purposes, and which were no longer needed, and to the elimination of ordinary reacting cows in the latter part of this period preparatory to the complete elimination of reactors.

4. The male calves sold for veal and as breeders rose in 1926 to 22 (the highest point reached up to that time) but the sale of producing cows was still low because it was highly desirable to retain all satisfactory cows. Seven poor producers, most of which had been held longer in the herd than they really should have been went to the butcher during this year.

5. In 1927 a new high record for values, namely, \$3,652.65, was established from a new high total of 38 animals sold. The shift to bull calves sold as breeders is noteworthy; this was made possible because there were so many good, normal calving cows. Since there was also a large number of surplus cows which were sold as producers, the sales netted an unusually good return.

### *Milk yield*

The year by year milk yield of the herd is given in table 3. An explanation appears desirable, however, before presenting and discussing this table.

The number of milk cows included in the second column of table 3 does not agree precisely with the record of breeding cows in table 1, largely because some grades were occasionally introduced into the herd temporarily for use in feeding experiments, and since neither they nor their descendants became a permanent part of the herd they were omitted in the consideration of milk yield. This did not apply, however, to a few grades that had been bred in the herd and whose yields are included.

When a cow had once freshened she was ever afterwards regarded as a milking cow so long as she remained in the herd, and no deduction was made for dry periods. The seeming low average number of months in milk as given in the third column is due to the continual shifting of the herd. When a cow was sold in the latter part of March, for instance, she was credited with three months in that particular year, regardless of her stage of lactation, and even if she was dry during a part of or all of that time.



Likewise, when a heifer freshened for the first time she was considered as a milk cow for just that fraction of the year that she was in milk; for example, if she calved during the first part of September she was recorded as being in milk four months in that year. If a heifer freshened for the first time before the tenth day of the month it was called a full month; if she freshened between the 10th and 20th of the month it was recorded as a half month; and if she freshened between the 20th and the last day of the month this last fraction of the month was not counted. Likewise, although in reverse order, a cow that was sold was similarly recorded.

Since the animals were of different ages, production records were converted to full age by using figures based upon the studies of Gowen (6), Ragsdale and Turner (7), and of Fohrman (8).

Also, certain animals were on Advanced Registry test and necessitated corrections for these records, but we were at a loss to find accurate conversion factors to apply to this herd. Eckles (9), in a study of Advanced Registry records made in 1922 in the herds of the University of Minnesota, the University of Missouri, the University of Nebraska and the Connecticut Agricultural College, found that the herd record was equivalent to about 58 per cent of the Advanced Registry record for the same cow, but, because of certain specifications as to his requirements, many of the cows with Advanced Registry records in the Connecticut herd were not included at that time; hence we do not believe that this figure applies to the whole herd. Woodward (10) reported a conversion factor of 66.67 per cent in the Government herd at Beltsville, where the cows were in box stalls while on test. Fohrman (11) claimed that an approximate increase of 11.5 per cent is obtained in a retest by virtue of the development of the cow. Riford (12), in a large commercial herd, found that increasing the number of milkings to three times a day resulted in an increase of about 15 per cent in the yield.

Only a very small number of the test cows in the Connecticut Agricultural College herd have been milked four times a day, and none throughout a complete registry record in recent years. Also, no cow has been held throughout the entire period in a box stall,

and the great majority have not been in a box stall at all except at calving time. Thus, it seems that in this herd the herd yield would be nearer 70 per cent of the Advanced Registry yield for all cows. This factor was used, therefore, in computing yields during the period when the cows were being milked more than twice daily. The number of cows maintained on Advanced Registry test has been fairly uniform.

TABLE 3  
*Production data presented by calendar years 1915-1927*

YEAR	NUMBER OF COWS	AVERAGE MONTHS IN MILK	ACTUAL AVERAGE YIELD PER COW		ACTUAL TOTAL MILK YIELD	CORRECTED FOR AGE AND ADVANCED REGISTRY YIELD PER COW		NUMBER OF 12-MONTH COW YEARS	12-MONTH COW YIELD FROM CORRECTED YIELD		PER CENT PREMATURE CALVINGS
			Milk	Fat		Milk	Fat		Milk	Fat	
					pounds						
1915	37	8.98	6,981	293.4	258,305	6,816	293.4	27.7	9,104	391.8	6.5
1916	39	7.70	5,765	232.0	224,828	5,516	226.5	25.0	8,591	352.8	10.5
1917	39	8.86	7,015	275.8	273,592	6,939	274.6	28.8	9,397	371.9	8.6
1918	34	8.15	6,279	258.0	213,471	6,195	258.3	23.1	9,127	380.4	5.7
1919	45	7.20	4,678	199.7	210,539	5,214	221.2	27.1	8,664	367.6	31.6
1920	48	8.24	4,917	201.2	236,009	5,169	217.9	33.0	7,528	317.0	36.4
1921	46	7.66	5,543	212.4	254,955	5,598	215.7	29.4	8,768	378.7	20.0
1922	41	8.13	6,309	246.3	258,682	6,315	246.3	27.8	9,316	363.3	15.8
1923	36	8.93	6,528	256.2	235,021	6,044	242.3	26.8	8,158	325.6	28.2
1924	47	7.19	6,503	259.2	305,663	6,099	254.8	28.2	10,108	425.2	15.4
1925	36	7.50	6,788	277.3	244,375	6,458	261.2	22.5	10,314	417.3	2.9
1926	48	7.49	7,200	278.8	343,578	7,064	271.2	30.0	11,314	434.5	6.2
1927	43	8.84	8,045	328.7	345,950	7,152	300.1	31.7	9,712	407.6	0.0

Table 4 is derived from table 3. Special attention is called to these data. The average fat production given in the column next to the last in table 3 is repeated in the second column of table 4. But the milk yield cannot be so satisfactorily presented, because the four breeds of cows in the herd are not maintained in the same numerical relationship from one year to another. Therefore, Table 4, in which the milk yield is calculated from the fat yield, gives the values in terms of milk yield for a Holstein, an Ayrshire, a Guernsey, and a Jersey herd.

Corrections have been made for differences in breed and in age, and for Advanced Registry test. There are still other variable factors for which there are no known means of correction. For instance, an effort has most naturally been made to improve the herd. No correction can be made for this difference, whatever it may be, but it can be said that there has been no bull in use at any period that has proved to be outstanding in transmission of yield qualities, and with four breeds involved no single bull has been able to create a dominant influence upon the whole

TABLE 4  
*Calculated milk yields from corrected per cow year fat yield*

YEAR	PER COW YEAR FAT YIELD FROM TABLE 3	EQUIVALENT MILK YIELD CALCULATED FROM FAT YIELD				PER CENT PREMATURE CALVINGS
		3.45 per cent milk	4 per cent milk	5 per cent milk	5.35 per cent milk	
		<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	
1915	391.8	11,357	9,795	7,836	7,323	6.5
1916	352.8	10,226	8,820	7,056	6,594	10.5
1917	371.9	10,779	9,298	7,438	6,951	8.6
1918	380.4	11,026	9,510	7,608	7,110	5.7
1919	367.6	10,655	9,190	7,352	6,871	31.6
1920	317.0	9,188	7,925	6,340	5,925	36.4
1921	378.7	10,977	9,468	7,574	7,078	20.0
1922	363.3	10,530	9,083	7,266	6,791	15.8
1923	325.6	9,438	8,140	6,512	6,066	28.2
1924	425.2	12,325	10,630	8,504	7,948	15.4
1925	417.3	12,096	10,433	8,346	7,800	2.9
1926	434.5	12,594	10,863	8,690	8,121	6.2
1927	407.6	11,861	10,190	8,152	7,619	0.0

herd. Furthermore it is admitted that it has been our aim to improve the feeding and management in the herd. During the past seven years the grain rations have been very much the same but pasture improvement and somewhat more liberal hay feeding, when pastures were short, have without doubt had some influence. However, the year to year yield seems to show no precise evidence of an influence from either of these factors.

The following deductions are made from tables 3 and 4.

1. The yield in 1915 was 391.8 pounds of fat per cow, equivalent to 9795 pounds of 4 per cent milk. The yield fluctuated

between this amount and 317.0 pounds of fat. In 1923 the yield was 325.6 pounds. There is not the slightest indication of an upward trend through these years that may be attributed to an improvement in breeding and management.

2. In 1924, when the aborting animals were promptly removed and the herd thus placed on a practically abortion-free basis, the average production was 425.2 pounds of fat, equivalent to 10,630 pounds of 4 per cent milk. Thus, for the first time the herd

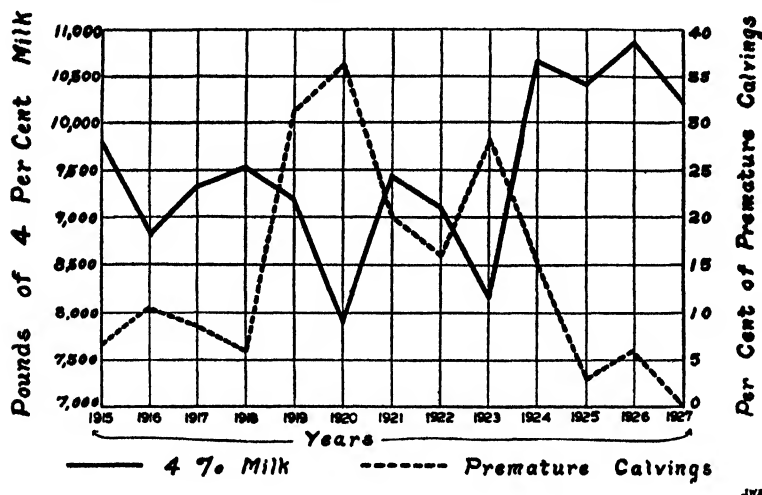


FIG. 1. ANNUAL PER COW MILK YIELD AND PREMATURE CALVING RECORD, 1915-1927

yield had gone beyond 400 pounds of fat and 10,000 pounds of 4 per cent milk.

3: From 1924 to 1927, inclusively, the yield has remained above 400 pounds of fat and 10,000 pounds of 4 per cent milk.

4. The equivalent milk yield of 3.45 per cent test reached 12,000 for the first time in 1924 and remained above this figure, except in 1927 when the yield was just below 12,000; the equivalent milk yield of 5 per cent test went above 8000 for the first time in 1924, and has remained above this figure; and the equivalent milk yield of 5.35 per cent test went above 8000 in 1926, and has been close to this level in each of the last four years.

5. For nine years before 1924 the average (direct average) was 361.0 pounds of fat. In the past four years the average has been 421.2 pounds of fat. For the nine years before 1924, the average yield per year of 4 per cent test milk was 9025, and in the past four years it has been 10,530 pounds.

6. Beginning in 1924, when all aborting animals were removed, and continuing through 1927, during the last three years of which the herd has been free from the Bang abortion disease, the herd has averaged 1505 pounds more per cow per year than during the previous nine years. This may at first thought appear to be a disappointing improvement, but it must be considered:

a. That during the nine years covered by these data all of the animals, of course, were not diseased.

b. Fifteen hundred pounds of milk, or 60.2 pounds of butterfat, represent the cream of the profit on a cow. At \$3.50 per hundred weight ( $7\frac{1}{2}$  cents per quart) this amount of milk is worth \$52.67. In a herd of 20 cows it has a value of \$1053.40.

#### *Other improvements*

One of the results of an abortion-free herd that has already appeared and which should be even more noticeable in another year or two is the improvement in the appearance and the soundness of the herd. It is no longer necessary to keep all cows simply because they will produce some milk. As a consequence, the herd is now more valuable than it has ever been and we believe it is destined to reach a still higher plane of production.

In addition, it is our belief that there has been some reduction in retained afterbirth and in the number of services per conception, but the differences may not be very pronounced, and more complete data are needed before these points are definitely determined. In an earlier publication (Bul. No. 135) it was pointed out that there was apparently not a great difference in favor of the non-reacting cows in these two respects.

#### SUMMARY

In looking forward, in 1923, to the establishing of an abortion-free herd at the Connecticut Agricultural College, the practice

was instituted of protecting maturing heifers by removing calves at six months of age, whether they were from abortion reacting or non-reacting dams, to separate premises a half mile distant from the main herd.

In order to stabilize the infection in the main herd the policy was adopted in 1924 of disposing of all aborting (premature calving) cows.

In February and March of 1925 the fifteen remaining reactors were removed from the herd and segregated. In July, 1925, one of the supposedly clean young cows reacted. She was promptly removed, and aborted two months later. Since that time not a single animal has reacted to the blood tests except young calves from the segregated reacting cows. This outstanding success in so completely removing the foci of infection in the initial separation was undoubtedly promoted by the process of stabilization or delimitation of the disease through the preliminary measures employed.

The abortion rate (premature calvings prior to the 265th day of gestation) which had varied in intensity during the years from 1904 to 1924, averaging 19.5 for each 100 calvings during this time, dropped to 2.9 per cent in 1925. It was 6.1 per cent in 1926, and 0.0 in 1927. The average premature calving rate during the past three years was 3.1 per cent, this figure being substantially the same as that previously reported by us from over 500 calvings of non-reacting cows.

The number of cows calving in each calendar year, which averaged 80.5 for each 100 cows from 1904 to 1924, was 87.4 in 1925, 100 in 1926, and 91.8 in 1927, the last three years being the period in which the herd has been free from abortion reactors.

The live stock sales reached a high peak in 1927, due not only to a larger number of surplus animals but also to the larger proportion of more valuable breeding and producing animals available from the non-reacting herd.

The milk yield of the non-reacting herd has averaged 1505 pounds more of milk testing 4 per cent per cow per twelve-month year than the previous herd which contained both reacting and non-reacting cows. During the last four years the average yield

of the herd has been 10,530 pounds of four per cent milk, while for nine years prior to that the average was 9025 pounds.

Finally, the appearance of the herd unquestionably shows an improvement as a result of the increased opportunity for culling that is afforded in an abortion-free herd.

In conclusion, the writers wish to express their appreciation to Dr. Charles A. Slanetz, Mr. George Brigham and Mr. Charles Oliver for their valuable assistance and coöperation.

#### REFERENCES

- (1) Storrs Experiment Station Reports: Bul. 93 (1918), 103 (1919), 108 (1921), 112 (1923), 123 (1924), 125 (1924), 130 (1925), 135 (1925), and 137 (1926), also *Jour. of Immunol.*, 1918, iii, pp. 389-393; 1920, v, pp. 399-416; and 1925, x, pp. 811-828.
- (2) THOMPSON, A. A. 1925 A study of abortion and experimental work at Oak Hill Estate. *Guernsey Breeders' Jour.*, p. 56, July 16.
- (3) SIMMS, B. T., AND MILLER, F. W. 1926 Practical results of attempts to control abortion disease. *Jour. Amer. Vet. Med. Assoc.*, lxvii, no. 4, January.
- (4) NEWSOM, I. E., AND CROSS, F. 1927 Control of abortion disease by blood test and segregation. *Colo. Agr. Exp. Sta. Bul.* no. 317.
- (5) BARNES, M. F. 1925 Bovine infectious abortion. *Penn. Dept. of Agr.*, viii, no. 8.
- (6) GOWEN, J. W. 1924 Milk Secretion. Williams & Wilkins, publishers. P. 55.
- (7) RAGSDALE, A. C., TURNER, C. W., AND BRODY, S. 1924 The relation between age and fat production in dairy cows. *Jour. Dairy Sc.*, vii, no. 2, p. 189.
- (8) FOHRMAN, M. H. 1926 Factors for adjusting milk and butterfat records of Register of Merit Jersey cows to a uniform age basis. *Jour. Dairy Sc.*, ix, no. 5, p. 469.
- (9) ECKLES, C. H. 1923 Dairy Cattle and Milk Production. Macmillan Company, publishers. P. 424.
- (10) WOODWARD, T. E. 1927 Influence of two planes of feeding and care upon milk production. *Jour. Dairy Sc.*, x, no. 4, p. 283.
- (11) FOHRMAN, M. H. 1926 Increased producing ability in dairy cows due to test conditions. *Jour. Dairy Sc.*, ix, no. 2, p. 215.
- (12) RIFORD, L. R. 1922 Two and three milkings daily. *Hoard's Dairyman*, lxxviii, p. 661.

## A DEFECT IN MILK DUE TO LIGHT\*

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In cold weather the "outdoor icebox" is used extensively, especially by apartment dwellers. Milk, with other food, is placed in a more or less open box on the window sill or even out on the sill. This milk sometimes develops an off flavor that has been described as a "cardboard" taste, for which the consumer is more likely to blame the milk distributor than himself.

The appearance of a cardboard flavor in milk is common and may be due to any of several causes. The term "cardboard" is itself indefinite and is undoubtedly applied to different flavors and odors which result from changes in the butterfat. Some flavors are designated as "flat" and others are slightly metallic, although most of the flavors which result from oxidation of the fat may be termed "tallowy" or "cardboard."

Hunziker (2), (3), has reviewed work by himself and others on the effect of various metals on the flavor of milk and other dairy products. He calls attention to the fact that metals from various parts of farm and milk plant equipment may enter the milk and act as oxidizers or catalyzers with the production of metallic and other off flavors. Flat or cardboard flavors may result in the action on milk fat.

Hammer and Cordes (1) have reported that the action of direct sunlight on milk and cream produces a definitely tallowy flavor on sufficient exposure and a distinct off flavor with less exposure. Off flavors were observed in certain milk samples after an exposure of only ten minutes; and tallowiness appeared after exposure for as short a time as forty-five minutes. Exposure to air apparently aided the development of the defect. The use of brown bottles was shown to prevent the appearance of the bad flavors, but the brown bottles had other disadvantages which more than offset

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this one advantage. Season of the year had little influence on the development of the flavor. Sunlight had a greater influence on milk of low butterfat content than on milk of high butterfat content.

It is generally supposed, however, that while direct sunlight is very active in causing these changes in milk, that diffuse light acts very slowly and may take days to bring about undesirable changes in flavor. In the experiments here reported the milk was never exposed to direct sunlight, but only to diffuse light in a north window which was never reached by the sun.

The defective taste which is being here discussed is not metallic but resembles cardboard, and the odor is like that of a drying linseed oil accompanied by a sweetish odor. As an after-taste there is in the back of the mouth a puckering sensation which is probably due to free fatty acids.

In this investigation it was apparent that light probably played a part in the production of the defect. To demonstrate that light was essential, in all the work described below, duplicate samples were prepared, of which one was exposed to daylight and the other was placed in the dark in a well-aerated metal container. In all cases the milk samples kept in the dark showed no evidence of a cardboard odor or flavor, even after seven to nine days at near freezing temperatures, whereas the samples kept in light at the same temperature developed the characteristic cardboard odor and taste after twenty to forty-eight hours of which eight to twenty-six hours were daylight. Exposure to diffuse daylight apparently instigates an oxidation process which continues in the dark. The samples were kept in flasks or bottles which were stoppered with cotton or covered with paper or a sterile glass beaker, so that no cardboard taste could come from a cardboard cap. The samples were never exposed to direct sunlight but were placed in a north window in diffuse light. Incubation temperatures were, for the most part, just above freezing. No difference in results was noted whether soft-glass milk bottles were used or hard-glass (Pyrex) flasks.

That the defect develops in the cream and not in the skim milk was shown by exposing skim milk, whole milk, and cream

from the same milk sample. The skim milk developed no cardboard taste, whereas the whole milk and cream showed the defect.

To determine whether the cardboard taste and linseed-oil odor might result from the absorption of part of the taste and odor of ordinary tallowy butterfat, the fat from one of the milk samples was separated and exposed to direct sunlight for a few hours. It was then held at 50°C. until a marked tallowiness developed when fresh skim milk was added and the mixture thoroughly shaken. The same odor and taste that was in the tallowy butterfat persisted in the reconstituted milk and no resemblance to the cardboard taste or linseed-oil odor was noted.

The effect of heating the milk on the development of the defect was shown by a comparison of raw samples and those pasteurized at 62.5°C. for one-half hour. The pasteurized samples usually developed the cardboard taste more rapidly than the raw samples. When the milk was heated in an Arnold steamer or was sterilized in the autoclave, the defect appeared a little more slowly. In the case of milk sterilized in the autoclave the action of both enzymes and bacteria was eliminated. These factors, however, may play a small part in the development of the defect in raw or pasteurized milk.

The resemblance of the odor of the milk with a cardboard taste to that of drying linseed oil indicated that the odor might be due to the oxidation of one of the more unsaturated fatty acids. Oleic acid, the chief unsaturated fatty acid of milk fat, is not so readily oxidizable as the more unsaturated linoleic and linolenic acids. It has been reported by Hunziker, Mills and Spitzer (4) that the characteristic chemical constants of milk fat vary when corn oil, linseed oil, and cottonseed oil are fed to cows, and that the iodine number, in particular, is increased by these feeds. Therefore it was thought that these oil feeds might so influence the milk fat that it would be more susceptible to oxidation. An experiment was conducted with the milk of cows selected from the experiment farm of the Bureau of Dairy Industry at Beltsville, Maryland. These cows were being fed as follows: Cows 1 and 2 received no oil feed; cows 3 and 4 received a heavy ration of cottonseed meal as their only oil ration; and cows 5 and 6 received

a heavy ration of linseed cake. The defect was found to be just as pronounced in the milk from cows 1 and 2, which received no oil feed, as in the milk from cows 3, 4, 5, and 6. The milk samples were obtained from the milk pails immediately after milking and did not pass over a cooler or through any other metal equipment.

#### DISCUSSION

The defect which has been described is apparently of very general occurrence for it developed in all milk samples from a number of sources. The remedy is to keep the milk in the dark even when the temperature is near freezing. Consumers should be so advised.

It is apparent that exposure to diffuse light for a few hours so shortens the induction period of the milk fat that oxidation may begin with consequent production of undesirable odors and flavors. The few hours of exposure to light apparently starts a process which continues in the dark and is accelerated by more daylight. Increased quantities of unsaturated fatty acids other than oleic acid seem unnecessary for the action. The odor and taste after the first twenty to forty-eight hours is that of an oxidized fat and gives no evidence of rancidity, although after exposure for a number of days rancidity may also begin to appear. Although the heavy glass of the milk bottles screens out the ultraviolet rays, it allows the passage of the active longer rays which exert a catalytic action. The milk fat apparently is made more easily oxidizable by the heat of pasteurization. The presence of a metal catalyst from equipment or other source would undoubtedly assist the catalytic action of daylight.

#### SUMMARY

A "cardboard" taste and "linseed-oil" odor develop in whole milk which has been exposed to diffuse daylight for eight or more hours at about freezing temperature. The light apparently acts as a catalyst in the oxidation of the milk fat. The defect develops more rapidly in pasteurized than in raw milk. The presence of neither enzymes nor bacteria is necessary for the

reaction. The defect develops no more rapidly in milk from cows fed heavy rations of cottonseed meal or linseed cake than in milk from cows receiving no oil feed.

## REFERENCES

- (1) HAMMER, B. W., AND CORDES, W. A.: A study of brown glass milk bottles. Research Bul. 64, Iowa Agric. Expt. Sta., (1920).
- (2) HUNZIKER, O. F.: Selection of metals in the construction of dairy equipment. Proc. World's Dairy Congress, 1923, 1189.
- (3) HUNZIKER, O. F.: The Butter Industry. 1927 ed., pp. 45, 182.
- (4) HUNZIKER, O. F., MILLS, H. C., AND SPITZER, G.: Moisture Control of Butter—I. Bul. 159, Ind. Agr. Expt. Sta., (1912).

# SOME OBSERVATIONS ON THE CONSISTENCY OF CREAM AND ICE CREAM MIXTURES\*

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## INTRODUCTION

In a previous paper Bateman and Sharp (3) gave the results of an investigation of the apparent viscosity of milk and some of the important factors which influence it. The object of this series of experiments was to study the "viscosity" of cream and ice cream mixtures in order to gain some idea of the reliability of the results obtained with viscometers which operate on the liquid with a single shearing force. A search of the literature showed that no work had been reported on the plasticity of cream or ice cream mixtures with the exception of a short paper by Masurovsky (9) who concluded that ice cream mixtures were plastic but that 20 and 40 per cent fat creams were not.

Bingham (4) has greatly advanced our knowledge of the laws of flow by showing the main difference between viscous and plastic flow through capillary tubes. The slightest pressure or force will cause the flow of viscous liquids although in some cases the flow will be extremely slow. The flow of viscous liquids is directly proportional to the force producing the flow. Plastic substances, on the other hand, have some of the properties of solids, that is, they are able to hold their shape under small shearing forces but are readily made to flow under higher shearing forces. The shearing force which is theoretically just necessary to start the flow of a plastic substance is called the "yield value." If at the higher forces producing the flow of a plastic substance, the yield value is subtracted from the total force then the flow of the plastic substance is directly proportional to this corrected force.

This relationship is brought out by equation (1) which is the

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equation for the determination of the consistency of a plastic substance by means of its flow through a capillary tube.  $\xi$  the consistency of a plastic substance corresponds to  $\eta$  the viscosity of a liquid.

$$\xi = \frac{\pi r^4 g (P - p) t}{8 l v} \quad (1)$$

Where  $r$  is the radius of the capillary,  $l$  its length,  $v$  the volume flowing through the capillary in the time  $t$ ,  $P$  the total pressure in grams per square centimeter producing the flow,  $p$  the yield value pressure and  $g$  the gravitational constant. The only difference between the fundamental equation for viscous flow and that for plastic flow is the substitution of  $(P - p)$  in the later for  $P$  in the former. It can readily be seen from equation (1) that if  $p$  equals zero then the substance is viscous. On the other hand if  $p$  has a value greater than zero the substance under investigation is plastic.

In studying the physical properties of milk, cream, or other colloidal suspensions, it is desirable to determine by means of observations made at several sufficiently high shearing forces, whether or not the coefficient of resistance to flow is independent of the force producing the flow. If the coefficient is found to be constant the material may be classed as truly viscous, if not, the material is only apparently viscous or may be truly plastic.

Bateman and Sharp (3) showed that even the viscosity coefficient of milk was dependent on the shearing force. This effect can be best brought out by reference to figure 2 of their paper in which the viscosity is plotted against the pressure producing the flow. It is seen that a truly viscous liquid such as the 20 per cent sucrose solution gives a viscosity-pressure curve that is a straight line parallel to the pressure axis; in other words the viscosity coefficient is independent of the pressure. This is not the case with milk since its viscosity coefficient shows a decrease as the force producing the flow is increased. This means that the so-called viscosity of milk cannot be expressed accurately by a single value.

A viscous liquid may be distinguished from a plastic substance by plotting the rate of flow through a capillary tube in cubic

centimeters per second at varying pressure. If the kinetic energy correction is negligible, the graph representing a viscous liquid will be a straight line passing through the origin. On the other hand if the straight portion of the line extrapolated intersects the pressure axis to the right of the origin as shown in figure 2 of this paper, the substance is plastic.

In this paper the rate of flow of cream and ice cream mixtures through a capillary tube was studied. It will be noted in figure 2 that at higher pressures, a straight line expresses the relation between the rate of flow and the pressure, and that this straight line extrapolated intersects the pressure axis to the right of the origin. The slope of the line times the constant of the plastometer gives the consistency, and the point of intersection on the pressure axis gives the yield value. At lower pressures, the pressure-rate of flow line curves towards the origin. This discrepancy is probably due to the partial breaking down of a structure in the dispersed phase and is overcome by using higher pressures, since under these conditions the relation between the rate of flow and shearing force becomes linear.

## EXPERIMENTAL

### *Apparatus*

The Bingham (4) plastometer has been used by various workers and the one used on this work was a modification of it. Figure 1 shows a diagram of the plastometer. It is similar to the one used by Sharp (11), except that the instrument was modified so as to better adapt it to the conditions of the problem.

The reservoir *A* consisted of a piece of glass tubing with an internal diameter of 2.5 cm., and a length of about 12 cm. It was closed at each end by means of one hole stoppers. A short piece of glass tubing *F*, was inserted into the top stopper and this served as a connection between the plastometer and the pressure line. Into the lower stopper, a capillary tube was introduced which projected through the rubber stopper *D*, into the receiving vessel *B*. The reservoir stoppers were firmly held in place by means of a clamp which consisted of two metal plates placed at

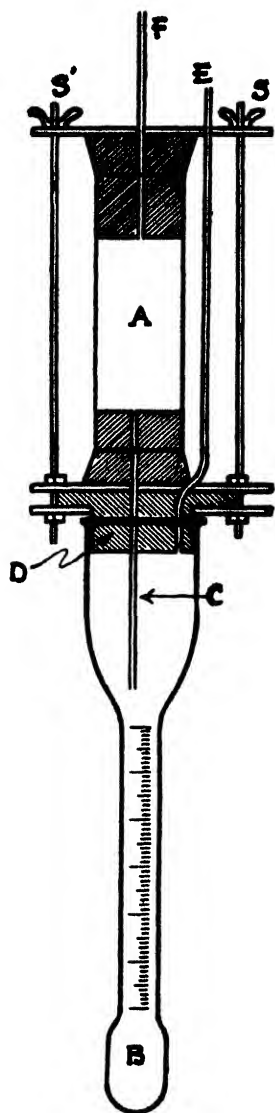


Fig. 1. DETAILS OF THE PLASTOMETER



the top and bottom of the tube *A*. These plates were kept in place by two bolts with winged nuts at the top. At the bottom was a metallic plate with a large hole in the center, through which the special rubber stopper *D* projected. The bolts pierced this plate, and firmly held the stopper *D* in position. The glass tube *E* served as an outlet for air which was displaced, when the liquid was forced from the reservoir *A*, through the capillary *C* into the receiving vessel *B*. This plastometer was substituted for the viscometer in the apparatus described by Bateman and Sharp (3).

*General procedure*

A definite amount of cream, which had been previously brought to the working temperature (25°C.) and thoroughly mixed, was poured into *A*. The stopper was then replaced and clamped in position. A receiving vessel *B* of desired volume was inserted over the special stopper *D*. The opening *E* was closed by means of a piece of rubber tubing and a clamp. This was done to prevent the leakage of cream into vessel *B*, before and after the determination. The completed apparatus was quickly inserted into the water bath and connected to the pressure line. The pressure, as indicated by the manometer, was adjusted prior to making the determination. The clamp was removed from *E*, and the pressure was turned on by means of the three-way stopcock. The cream was allowed to flow until it filled the receiving vessel *B* somewhere along the constricted, graduated portion. When the cream was thin, or when higher pressures were used, a receiver having a larger volume was attached to the apparatus. This made it possible to allow more cream to flow through the capillary, and thereby, kept the length of the time of flow within desirable limits. At the end of the run, the stopcock was turned so as to connect the plastometer with the atmosphere of the laboratory. When the pressure was released from the instrument, the watch was stopped and the time of flow *t*, recorded. The volume of flow *v*, was noted directly from the graduations on the receiver.

*Calibration*

It was first necessary to select a number of capillary tubes of uniform bore, cut these into appropriate lengths, and determine by trial runs which one was best suited to the properties of the samples of cream to be studied. The radii of the capillaries were measured by the microscopical method. These measurements were checked by filling the bore of the capillary with mercury, and weighing. From the weight and length of the mercury thread, the radius of the capillary was calculated. Bingham (4) fully described this method.

The radius of the capillary used in this work was found to be 0.02678 cm. with the microscope, and 0.02675 cm. with the mercury thread, which gives an average of 0.02676 cm. This capillary was next ground down until its length was equal to 10 cm.

In the introductory part, it was noted that the rate of flow of plastic substances might be approximately expressed by equation (1).

It is usually customary to express the experimental data in terms which are supposedly independent of the dimensions of the capillary. The pressure,  $P$ , and the yield value,  $p$ , were changed to dynes per square centimeter, and are represented by  $F$  and  $f$ , respectively. The value of  $F$ , or the shearing force on the walls of the capillary is given by the following equation:

$$F = \frac{\tau P g}{2 l} \quad (2)$$

Substituting the values from (2) into equation (1) we obtain:

$$\xi = \frac{\pi r^3 (F - f)}{4 V} \quad (3)$$

Where  $V$  is the flow in cubic centimeters per second, but, for the same capillary

$$\frac{\pi r^3}{4} = C \text{ (constant)} \quad (4)$$

which was numerically equal to  $1.505 \times 10^{-5}$  for the capillary used here.

Substituting this value of  $C$  into equation (3) gives the working equation for these experiments.

$$\dot{\epsilon} = \frac{1.505 \times 10^{-3} (F - f)}{V} \quad (5)$$

In plasticity measurements the kinetic energy corrections are usually omitted, since they are ordinarily negligible under the experimental conditions used for studying plasticity.

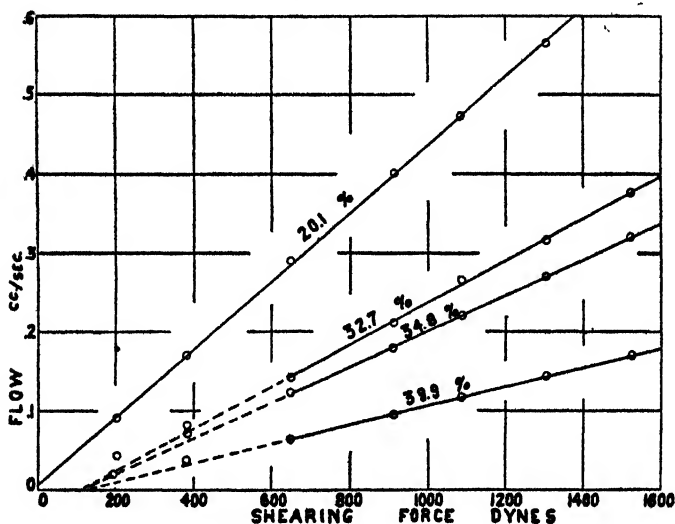


FIG. 2. THE RATE OF FLOW OF PASTEURIZED CREAM OF VARYING FAT CONTENT UNDER VARYING SHEARING FORCE

The total pressure,  $P$ , which was being used in any given determination of plasticity, was found by adding the pressure due to the average hydrostatic head of the cream in the plastometer to that indicated by the manometer. The pressure in each case was then changed to the shearing force in dynes by multiplying by the factor 1.312 obtained by substituting the values for  $r$ ,  $g$ , and  $l$  in equation (2).

The yield value,  $f$ , was determined by the method of least squares, and checked graphically.

*Effect of fat content and aging on the plasticity of pasteurized cream*

The results obtained are given in table 1. The effect of increasing fat content on the plasticity constants is expressed graphically in figure 2. It is seen that as the fat content increases so also does the yield value and the consistency. The sample containing 20.1 per cent fat in the figure apparently gives a small negative yield value. This was due to the fact that the rate of flow was so great that the kinetic energy correction which was assumed to be negligible actually exerts an appreciable effect. If a correction is made for kinetic energy a small positive yield value is obtained. This was the only sample which did not show a definite positive yield value in the graph. The data for the sample containing 34.8 per cent fat was taken from another experiment in which case the cream was pasteurized twice. As the fat concentration increases the yield values are 8, 110, 109, and 120 while the consistency values are 3.21, 5.64, 6.66, and  $12.4 \times 10^{-2}$ . The last three yield values are very nearly the same while the consistency of the same samples increases considerably. Since these were samples of pasteurized cream, the fat globules were probably not clumped to any considerable extent and more reproducible results could be obtained than with raw cream.

Various investigators have shown that pasteurized cream increases only slightly in viscosity on aging. The effect of aging on two samples is shown in table 1. Aging caused a slight increase in both the yield value and in the consistency.

*Effect of heating on the plasticity of cream*

Woll (16) and Babcock and Russell (1) (2) have shown that heating cream decreases the viscosity and breaks up the clusters of fat globules. Babcock and Russell attributed the decrease in viscosity to the breaking up of the clumps of fat globules. In our experiment 44.9 per cent fat cream was heated at 73°C. for thirty minutes. This temperature was chosen because such heating destroys the clumping of the fat globules while at the same time leaves the plasma with the same viscosity as unheated

TABLE 1

*Effect of fat content and aging on the consistency of pasteurized cream determined at 25°C.*

SAMPLE NUMBER	DAYS OF AGING	FAT CONTENT	EMULSIFYING FORCE DYNES (F)	YIELD VALUE (f)	(F-f)	FLOW V	CONSISTENCY $\frac{C(F-f)}{V}$
		per cent				cc. per second	
1†	0	20.1	1,305 1,087 912 649 383 205	8	1,297 1,079 904 641 375 197	0.566 0.473 0.401 0.290 0.171 0.092	$3.20 \times 10^{-3}$ 3.22 3.22 3.20 3.22 3.19
Average .....							3.21
2	0	32.7	1,522 1,306 1,087 911 651 383* 206*	110	1,412 1,196 977 801 541 273 96	0.376 0.316 0.266 0.213 0.143 0.083 0.044	$5.65 \times 10^{-3}$ 5.70 5.52 5.66 5.69 4.96 3.28
Average .....							5.64
2	4	32.7	1,522 1,305 1,087 911 649 383* 204*	124	1,398 1,181 963 787 525 259 80	0.318 0.263 0.216 0.177 0.120 0.071 0.037	$6.62 \times 10^{-3}$ 6.76 6.71 6.69 6.59 5.48 3.27
Average .....							6.67
3	0	39.9	1,524 1,307 1,089 912 649 383* 196*	120	1,404 1,187 969 792 529 263 76	0.171 0.145 0.117 0.096 0.065 0.038 0.021	$12.4 \times 10^{-3}$ 12.3 12.5 12.4 12.3 10.4 5.7
Average .....							12.4
3	3	39.9	1,503 1,305 1,087 912 649* 384*	184	1,319 1,121 903 728 465 200	0.153 0.130 0.103 0.083 0.055 0.033	$13.0 \times 10^{-3}$ 13.0 13.2 13.2 12.7 9.1
Average .....							13.1

\* Not used in the average or in determining the yield value.

† Corrected for kinetic energy.

plasma as was shown by Whitaker, Sherman and Sharp (14). The yield value before heating was 262 dynes and the consistency was  $37.9 \times 10^{-2}$ , after heating the yield value was 226 dynes and the consistency was  $26.4 \times 10^{-2}$ . Heating under these conditions decreased both the yield value and the consistency, the latter decreasing the most. This decrease in plasticity must be attributed to an effect of the fat; mainly a less clumped condition of the fat globules.

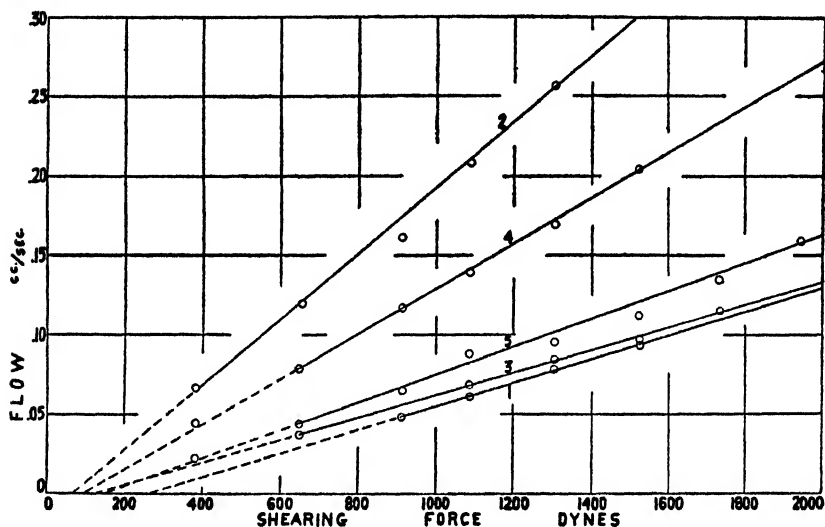


FIG. 3. THE RATE OF FLOW OF DIFFERENT ICE CREAM MIXTURES UNDER VARYING SHEARING FORCE

There is little doubt that the effect of heating would have been more pronounced if the plasticity constants had been determined at a lower temperature.

#### *Effect of homogenization on the plasticity of pasteurized cream*

Buglia (5) and Bateman and Sharp (3) have shown that homogenization increases the viscosity of whole milk while it produces no appreciable effect on the viscosity of skim milk. Wiegner (15) also showed that the viscosity of whole milk was

TABLE 2  
*Consistency of several ice cream mixtures at 25°C.*

ICE CREAM MIXTURE NUMBER	SHEARING FORCE DYNES (F)	YIELD VALUE (f)	(F-f)	FLOW $\dot{\gamma}$ cc. per second	CONSISTENCY $\frac{C(F-f)}{\dot{\gamma}}$
1	1,524	264	1,260	0.0931	$20.4 \times 10^{-3}$
	1,308		1,044	0.0772	20.4
	1,089		825	0.0608	20.4
	914		650	0.0481	20.3
	Average.....				20.4
2	1,307	66	1,241	0.257	$7.27 \times 10^{-3}$
	1,089		1,023	0.208	7.40
	913*		847	0.162	7.87
	654		588	0.119	7.44
	383		317	0.067	7.12
	Average.....				7.31
3	1,737	126	1,611	0.1154	$21.0 \times 10^{-3}$
	1,523		1,397	0.0972	21.6
	1,307		1,181	0.0837	21.2
	1,088		962	0.0680	21.3
	649		523	0.0375	21.0
	Average.....				21.2
4	1,524	93	1,431	0.204	$10.6 \times 10^{-3}$
	1,307		1,214	0.169	10.8
	1,088		995	0.139	10.8
	913		820	0.117	10.6
	649		556	0.079	10.6
	383*		290	0.0448	9.7
	Average.....				10.7
5	1,948	150	1,798	0.151	$18.9 \times 10^{-3}$
	1,735		1,585	0.135	17.7
	1,522		1,372	0.112	18.4
	1,305		1,155	0.095	18.3
	1,088		938	0.078	18.1
	912		762	0.064	17.9
	649		499	0.0434	17.3
	383*		233	0.0225	15.6
	Average.....				18.1

\* Not included in the average or in determining the yield value.

increased by homogenization. Bateman and Sharp (3) found that the fat globules in homogenized milk were not clumped. Mortensen (10) and Sherwood and Smallfield (13) stated that the fat globules in homogenized cream were clumped and the latter concluded that the extent of the clumping was related to the viscosity.

The effect of homogenization on the plasticity constants of a sample of 34.8 per cent fat cream was determined. The yield value before homogenization was 109 dynes and the consistency was  $6.66 \times 10^{-2}$ , and after homogenization the yield value increased to 336 dynes while the consistency increase to  $52.2 \times 10^{-2}$ . Homogenization increased the yield 3.1 times while it

TABLE 3

*Approximate composition of the ice cream mixtures of table 2*

MIXTURE NUMBER	FAT	MILK-SOLIDS —NOT-FAT	SUGAR	GELATINE	YIELD VALUE	CONSISTENCY
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		
1	11	11	14	0.3	264	$20.4 \times 10^{-2}$
2	11	6	14	0.3	66	7.31
3	11	11	18	0.3	126	21.2
4	11	11	10	0.3	93	10.7
5	12	11	14.5	0.4	150	18.1

increased the consistency 7.8 times. Thus homogenization produces a tremendous increase in the resistance of flow.

#### *The plasticity of ice cream mixtures*

The plasticity constants for 5 different ice cream mixtures are presented in table 2 and figure 3. The composition of the mixtures is given in table 3. The compositions given in table 3 are only approximate and the previous treatment of the mixtures is not known so that no attempt should be made to correlate the plasticity constants with the composition of the ice cream mixtures. The data are presented to show the relative magnitude of plasticity constants which might be expected for ice cream mixtures. The plasticity data given for these ice cream mixtures corresponds to what Leighton and Williams (7) would probably call "apparent viscosity" at 25°C.



## DISCUSSION

The plasticity experiments reported in this investigation were all carried out at 25°C. and not at near the freezing or storage temperature, because the temperature factors of the measurement could be controlled more accurately at 25°C.

The graph in figure 2 indicates that 20.1 per cent fat cream is viscous since the yield value was apparently zero. This is not actually true but appears so because the rate of flow was so great that the kinetic energy error was appreciable. After making this correction 20 per cent cream shows a small yield value and is therefore plastic. The capillary used was chosen because it could be used with the more plastic samples and therefore it was not suited for the measurement of the flow constants of the 20 per cent pasteurized cream. The possible effect of the diameter of the capillary on the plasticity constants was not determined.

The expected influences of aging, heating, and homogenization were found.

The so-called "body" or "viscosity" of cream and ice cream mixtures has received much attention. Various methods have been used to measure this property and the results obtained have not always shown the expected relation to other properties of the cream or ice cream mixture. Many attempts have been made to correlate the freezing properties of ice cream mixtures with their viscosity. Some investigators believed that they found such a relation while others did not.

The results presented here show that what has usually been called the viscosity of cream or ice cream mixtures is made up of two factors which do not necessarily run parallel with each other. Investigators have taken some unknown relation between these two factors and have tried to relate it to the properties of the cream or ice cream mixture. In their investigations the results have been expressed as if the yield value were zero which this investigation shows is an unwarranted assumption in most cases. The character of this error can be easily shown with our data by assuming that the yield value is zero in all

cases and then calculating the results on that basis. As can readily be understood from an observation of the graphs which show a definite yield value, the so-called viscosity values will be higher the lower the shearing force at which the measurement is made. In order to bring out this effect more clearly and to

TABLE 4

*A comparison of the plasticity constants with the "viscosity values" which would have been obtained at 1500, 1000, and 500 dynes shearing force, if the samples were considered to be viscous instead of plastic*

SAMPLE ORDER NUM- BER	DESCRIPTION	PLASTICITY CONSTANTS		"VISCOSITY" AT VARI- OUS SHEARING FORCES		
		Yield value	Consistency	1500 dynes	1000 dynes	500 dynes
1	Pasteurized 20.1 per cent fat cream fresh	8	$3.21 \times 10^{-3}$	cp. 3.47	cp. 3.45	cp. 3.40
2	Pasteurized 32.7 per cent fat cream fresh	110	5.64	6.10	6.35	7.17
3	Pasteurized 34.8 per cent fat cream fresh	109	6.66	7.19	7.49	8.55
4	Pasteurized 32.7 per cent fat cream aged	124	6.67	7.24	7.60	8.85
5	Ice cream mixture no. 2	66	7.31	7.60	7.80	8.46
6	Ice cream mixture no. 4	93	10.7	11.2	11.8	13.0
7	Pasteurized 39.9 per cent fat cream fresh	120	12.4	13.4	14.1	16.4
8	Pasteurized 39.9 per cent fat cream aged	184	13.1	15.0	16.0	20.3
9	Ice cream mixture no. 5	150	18.1	19.0	20.1	24.3
10	Ice cream mixture no. 3	126	21.2	23.0	24.3	27.9
11	Ice cream mixture no. 1	264	20.4	24.5	27.4	41.8
12	Heated 44.9 per cent fat cream	226	26.4	31.0	34.0	47.9
13	Raw 44.9 per cent fat cream	262	37.9	45.8	51.2	79.2
14	Pasteurized 34.8 per cent fat cream homogenized	336	52.2	68.4	78.2	150.0

make the values comparable, the flow in cubic centimeters per second at the shearing forces of 1500, 1000, and 500 dynes was determined graphically and these values were substituted in equation (5). The results obtained are given in table 4. The data are arranged in ascending order of viscosity at 1500 dynes

shearing force. This procedure of recalculating the data gives the same kind of results as would be expected if the viscosity of the same set of samples was determined at the same time, in three different viscometers of widely varying shearing forces. The comparison made here is perhaps a little more justified since the various values were all obtained with the same instrument operated with different shearing forces.

The results in table 4, with the exception of sample 1, show that if the samples are assumed to be truly viscous the viscosity in centipoise is higher the lower the shearing force applied. In some cases the differences are quite large, for example sample 14 gave a viscosity of 68 centipoises at 1500 dynes and 150 centipoises at 500 dynes, or an increase of 120 per cent. The differences would be greater if a shearing force still lower had been used. Sample number 14 is about 20 times as viscous as sample number 1 at 1500 dynes and 44 times as viscous at 500 dynes.

The difficulties in finding a relation between the viscosity of ice cream mixtures and their freezing properties is shown by comparing samples 10 and 11. At 1500 dynes sample 11 is 6 per cent more viscous than sample 10; at 500 dynes it is 46 per cent more viscous, and the consistency of sample 10 is about 5 per cent greater than sample 11. Furthermore the samples are not all arranged in the same order of increasing viscosity as a comparison of samples 3, 4, and 5 shows.

While much useful and valuable information may result from single "viscosity" determinations yet, these few considerations indicate the difficulties which may arise in finding a simple relation between a single viscosity measurement and the other properties of cream or ice cream mixtures. Considerable time is required to determine the pressure-flow relationship for a single sample. This is a great handicap in routine testing. On the other hand the so-called viscosity results obtained at some single unknown shear gradient may be subject to great difficulties of interpretation since another viscometer might possibly arrange some of the samples in a different order of increasing viscosity and might produce widely different spreads between the results obtained. The results presented by Morten-

sen (10) bring out this last point. He gives the relative viscosity of raw 35 per cent fat cream as 1.57, with water as 1.00, a value considerably lower than the average value obtained by Kobler (6) for whole milk. The relative viscosity of the ice cream mixtures which Mortensen investigated ranged from 1.22 to 2.12.

Another difficulty enters in the study of suspensions. The viscosity-concentration relationship studied at a single shearing force may be such that if a series of samples is arranged in the order of increasing viscosity at one concentration, the order may be quite different at another concentration. Sharp and Gortner (12) have shown this to be true with flour-in-water suspensions. The results obtained by Leighton and Williams (7) (8) indicate that it might also be true with ice cream mixtures. Leighton and Williams (8) give calculations of the viscosity of the unfrozen part of the ice cream mixture during the freezing process. Their results on the effect of temperature lead one to suspect that occasionally at least one might find samples which would arrange themselves in one order of increasing viscosity at 0°C. and in an entirely different order at the lower temperatures where the actual freezing is taking place. The fact that the presence of ice in the mixture increases the viscosity or really the plasticity of the mixture tremendously must also be borne in mind.

The work of Leighton and Williams and the results presented here indicate that if a thorough study of the "viscosity" of ice cream mixtures in relation to their other properties is to be made, the rate of flow should be determined at various pressures for each of several temperatures and several concentrations. Adopting the terminology of Leighton and Williams (7), they should also be studied in connection with their apparent (before agitation) and basic (after vigorous agitation) plasticity. This would be a considerable task.

#### SUMMARY

1. The yield value and consistency of cream increased with fat content, aging, and homogenization and decreased by heating.
2. Ice cream mixtures of average composition were shown to vary considerably in yield value and consistency.

3. Some of the difficulties which may arise if plastic substances are treated as viscous were shown.

4. Attention is called to some of the difficulties in relating the "viscosity" of ice cream mixtures to their freezing properties.

#### REFERENCES

- (1) BABCOCK, S. M., AND RUSSELL, H. L. 1896 Conditions affecting the consistency of milk. *Wisc. Agr. Expt. Sta. 13th Ann. Rpt.*, p. 73-80.
- (2) BABCOCK, S. M., AND RUSSELL, H. L. 1896 On the restoration of the consistency of pasteurized milk and cream. *Wisc. Agr. Expt. Sta. 13th Ann. Rpt.*, p. 81-94.
- (3) BATEMAN, G. M., AND SHARP, P. F. 1928 A study of the apparent viscosity of milk as influenced by some physical factors. *Jour. Agr. Research*, xxvi, 647-674.
- (4) BINGHAM, E. C. 1922 *Fluidity and Plasticity*. McGraw-Hill Book Company, New York.
- (5) BUGLIA, G. 1908 Ueber einige physich-chemische Eigenschaften der homogenisierten Milch. *Kolloid Z.*, ii, 353-354.
- (6) KOBLER, B. 1908 Untersuchungen über Viskosität und Oberflächenspannungen der milch. *Archiv. f. ges. Physiol.*, cxxv, 1-72.
- (7) LEIGHTON, A., AND WILLIAMS, O. E. 1927 The basic viscosity of ice-cream mixes. *Jour. Physical Chem.*, xxxi, 596-600.
- (8) LEIGHTON, A., AND WILLIAMS, O. E. 1927 The effect of temperature on the basic viscosity of ice-cream mixes. *Jour. Physical Chem.*, xxxi, 1663-1668.
- (9) MASUROVSKY, B. I. 1925 Which is it: Viscosity or plasticity? *Ice Cream Trade Jour.*, xxi, no. 5, 61-62.
- (10) MORTENSEN, M. 1918 Factors which influence the yield and consistency of ice cream. *Iowa Agr. Expt. Sta. Bul. No. 180*.
- (11) SHARP, P. F. 1926 Wheat and flour studies. V. Plasticity of simple flour-in-water suspensions. *Cereal Chem.*, iii, 40-56.
- (12) SHARP, P. F., AND GORTNER, R. A. 1923 Viscosity as a measure of hydration capacity of wheat flour and its relation to baking strength. *Minn. Agr. Expt. Sta. Tech. Bul. No. 19*.
- (13) SHEERWOOD, F. F., AND SMALLFIELD, H. L. 1926 Factors influencing the viscosity of cream and ice cream. *Jour. Dairy Sci.*, ix, 68-77.
- (14) WHITAKER, R., SHEERMAN, J. M., AND SHARP, P. F. 1927 Effect of temperature on the viscosity of skimmilk. *Jour. Dairy Sci.*, x, 361-371.
- (15) WIEGNER, G. 1914 Ueber die Aenderung einiger physikalischer Eigenschaften der Kuhmilch mit der Zerteilung ihren dispersen Phasen. *Kolloid Z.*, xv, 105-123.
- (16) WOLL, F. W. 1895 The effect of pasteurization and sterilization on the viscosity and fat globules of milk and cream. *Wisc. Agr. Expt. Sta. 12th Ann. Rpt.*, p. 164-173.

# A STUDY OF THE "COMMON WHITE" YEASTS FOUND IN DAIRY PRODUCTS\*

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At the present time a great deal of investigation is centered on the microflora of dairy products. At first investigators put much emphasis on the bacteria alone, but now attention has been turned to the yeast content of these products. Yeasts are often mentioned in reporting the microflora of dairy products without reference to any particular species or types. It was the purpose of this study to describe more in detail the so-called "common white" yeasts found in dairy products. These include the yeasts that produce typical, regularly circular, whitish, glistening, convex colonies with an entire edge and do not ferment lactose. These yeasts are distinguished from the chromogenic type by their lack of color and from the mycoderma and rapid liquifiers by the type of colony. The colonies of the "common whites" are much the same as those of the lactose fermenters, but the production of acid and gas in milk by the latter serves to readily distinguish the two. In addition to the study of the description some emphasis was put on the classification of the "common whites" and special note as to whether they might be of any importance in the normal processes or changes that take place in dairy products.

Hammer (1) suggested the name of *Torula lactis-condensi* for these yeasts in his studies on formation of gas in sweetened condensed milk. Cordes (2) isolated a number of this type of yeast in his study of yeast forms present in milk and milk products. Cordes and Hammer (3) refer to the "common white" yeasts as group IV, in studying the yeasts found in dairy products. They also state that it is a catchall group which further study will be certain to divide up on a more definite basis.

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Samples of milk, butter, cream, and allied dairy products were plated with whey agar, using 1 cc. of 2 per cent solution tartaric acid to keep down the bacteria. At times difficulty was encountered on account of excessive mold growth on the plates, but this was remedied by using whey agar containing 4 per cent NaCl. After forty-eight hours, at room temperature, the plates were examined for yeast colonies. The low power microscope was used to identify the colonies.

Purification was accomplished by repeated plating. After purification the yeasts were studied in much the same way that bacteria from dairy products are studied. Spore production was determined by staining whey agar cultures one to seven months old. Whey gelatin stab cultures were also stained for spores. Acid and gas production was studied in milk and 2 per cent sugar bouillons "Giant" colonies were also observed.

One hundred sixty cultures from cream, butter, soft cheese, milk and other minor sources were studied. The morphology of the cultures was given rather careful study, but it was very difficult to divide the yeasts definitely on this basis, because there was so much variation in form and size of cells. Quite definite results were obtained by different growth temperatures. Some of the cultures grew well both at room temperature and at 37°C., other cultures grew well at room temperature but poorly or not at all at 37°C. Three very distinct changes were brought about by inoculating into litmus milk, which were of much value in dividing the organisms into groups:

1. Sweet curdling of the litmus milk in ten to fifteen days, and a partial digestion of the casein in about thirty days.

2. Producing alkalinity in litmus milk in ten to fifteen days, the alkalinity increasing with age.

3. No change in litmus milk. Even after thirty days the litmus milk would appear the same as at the time of inoculation, yet slides made from the litmus milk cultures revealed that cells were present.

Chiefly on the basis of morphology, growth temperatures, and the action of litmus milk, the author concluded that the organisms

naturally divided into four types. Other characteristics which were not so outstanding seemed to substantiate this division.

It is very evident that "common white" yeasts are very numerous in dairy products. This type of yeast can be isolated from many different types of dairy products.

This type of yeast is also very resistant. The entire hundred and sixty cultures were left in the refrigerator for a period of over ten months at a temperature that varied from 0° to 10°C. After this storage period the cultures were still alive.

Spore formation did not seem to be very prevalent and seemed to depend to a large extent on the conditions under which the cultures were kept.

There is one characteristic of the "common white" yeasts that seems to be constant and that is the yellow coloration when the colonies become contaminated with *Aspergillus niger*. This yellow pigment formation was thought to be a possible differentiation of the groups; however, this proved to be a general characteristic and did not serve as a guide to division.

*I. Type A.* Large, rapid growing oval or elliptical yeast which grew well at both room temperature and 37°C. Sweet curdled litmus milk in ten to fifteen days, and partly digested the casein in thirty days.

*II. Type B.* Large, rapid growing, oval yeast with some elongated cells, which grew well at room temperature and very poorly, or not at all at 37°C. Produced no change in litmus milk.

*III. Type C.* Medium sized, rapid growing, oval yeast with some elongated cells, which grew both at room temperature and 37°C. Produced alkalinity in litmus milk in ten to fifteen days, the alkalinity increasing with age.

*IV. Type D.* Medium sized oval cells with some cells nearly round. No growth at 37°C. Produced alkalinity in litmus milk in ten to fifteen days, the alkalinity increasing with age.

The action of the "common white" yeasts is slow. It is concluded from this and their general behavior that they do not have much influence on the changes brought about in dairy products by microorganisms.



## REFERENCES

- (1) HAMMER, B. W. 1919 Studies on Formation of Gas in Sweetened Condensed Milk. Iowa Station Res. Bul. 54.
- (2) CORDES, W. A. 1920 A Study of Yeast Forms Present in Milk and Milk Products. Thesis for Master's Degree, Iowa State College.
- (3) CORDES, W. A., AND HAMMER, B. W. 1927 Studies on Yeasts in Dairy Products. Jour. of Dairy Sc., x, no. 1.

# THE INFLUENCE OF THE pH OF AGAR MEDIA UPON THE BACTERIAL COUNTS OF RAW AND PASTEURIZED MILK\*

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A study of this subject was suggested by a statement in the American Public Health Association's publication entitled, "Standard Methods of Milk Analysis" (fourth edition, 1923, page 5) which is as follows:

A medium consisting of the above ingredients, including a suitable peptone, ordinarily has a reaction between  $\text{pH} = 6.2$  and  $7.0$ . If within these limits, the reaction requires no adjustment for milk analysis. The most desirable reaction is about  $\text{pH} = 6.5$  to  $6.6$ , but any reaction between  $\text{pH} = 6.2$  and  $7.0$  is allowable. No change in reaction should be made without carefully determining the H-ion concentration of the finished medium by the method described below.

The specific problems, therefore, are, first, to find whether or not there is a material variation in bacterial counts when beef extract agar is employed with the following pH values— $6.2$ ,  $6.4$ ,  $6.6$ ,  $6.8$ , and  $7.0$ —for plating raw milk. Second, to find the most satisfactory pH value when beef extract agar is employed with the following pH values— $6.2$ ,  $6.4$ ,  $6.6$ ,  $6.8$ , and  $7.0$ —for plating pasteurized milk.

The raw milk samples were taken from milk shipped to the University of Maryland Dairy. The individual shippers were selected because of their sanitary methods. Samples were taken both from the milk of individual shippers and from the pooled milk of several shippers.

A portion of the pasteurized milk samples were collected just after the milk had been cooled, others were taken from milk which had been held in the ice box for five or six hours at temperatures varying from  $34^{\circ}$  to  $40^{\circ}\text{F.}$ , while still other samples

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were taken from milk which had been carried on trucks over a milk route, and returned to the refrigerator.

The directions given in the before mentioned edition of Standard Methods of Milk Analysis were followed in the preparation of the media, plating, incubation, and in counting.

The experiment was started about the first of October and ran through the month of March. One hundred samples each of raw and pasteurized milk were examined. Five samples of raw and five of pasteurized milk were plated, in dilutions of 1:100, 1:1,000, and 1:10,000 each week.

Agar of pH values of 6.2, 6.4, 6.6, 6.8, and 7.0 were prepared every two weeks for plating this milk. The reaction was deter-

TABLE 1  
*Influence of pH of agar media upon bacterial counts of milk*

pH	RAW MILK		PASTEURIZED MILK	
	Average count, 99 samples	Mean variation	Average count, 100 samples	Mean variation
6.2	51,455	±6,824	30,342	±3,575
6.4	52,797	±7,911	31,293	±3,585
6.6	53,103	±7,621	30,634	±3,413
6.8	53,210	±8,491	30,396	±3,501
7.0	50,739	±6,608	29,857	±3,521

mined by the colorimetric method, using the LaMotte 3 B. set and following the procedure recommended by its manufacturers. The indicator, brom thymol blue, with a range of 6.0 to 7.6 was used. After the pH was determined, the agar was adjusted to the desired point.

It is well known that media does not always show an increase in titrable acidity after autoclaving. To correct for such a change, a sample was autoclaved to determine the drop and enough alkali was added to make up for any deficiency. Most of the media made showed an initial titration of from 6.0 to 6.2. The media was then tubed and autoclaved at 15 pounds pressure for twenty minutes, cooled and placed in the ice box until used.

Table 1 shows the data.

The results obtained by plating 99 samples of raw milk showed but little variation in counts. The medium having a hydrogen ion concentration of 6.8 gave the highest average bacterial count. The other media ranged as follows: 6.6, 6.4, 6.2, and 7.0.

The results obtained by comparing the results of one hundred samples of pasteurized milk plated on beef extract agar, titrated to the before mentioned pH's, like raw milk, showed but little variation. The medium having a hydrogen ion concentration of 6.4 gave the highest average bacterial count, the others ranged in the following order: 6.6, 6.8, 7.0, and 6.2.

#### CONCLUSION

After a study of the available literature, together with the foregoing data, the following conclusion would seem warranted:

If the bacterial counts of raw and pasteurized milk plated on beef extract agar are significantly affected by the hydrogen ion concentration of the media when ranging between 6.2 and 7.0, the 199 samples are not sufficient to show this significance.

# THE BACTERIAL CONTENT OF ORANGE SHERBET\*

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A great deal has been said and written about the increase in the consumption of ice cream during the past two decades; less publicity, however, has been given to the increasing popularity of sherbets and ices. It is quite generally agreed that the increase in demand has been phenomenal, but there are no statistics available in this country which show the amount of these products manufactured each year.

It has been claimed that the increased demand for the lighter frozen desserts has been due to the modern fad of dieting, nevertheless, it must be recognized that there has been a marked improvement in the flavor and texture of these delicacies which has elicited public attention and favor. Likewise, it must be recognized that development of the public confidence in ice cream as a safe, wholesome, and sanitary food has had a marked effect on the sale of frozen desserts in general.

In view of the fact that ices and sherbets for the most part are made from ingredients usually considered relatively free from bacteria, very little attention has been paid to their sanitary control. Ices usually contain water, sugar, gelatin, eggs, and either fresh or preserved fruits, whereas the sherbets have in addition a greater or less amount of ice cream mix or dairy products. One would expect the water, sugar, eggs, and fruit to contain very few bacteria. Most of the gelatin (1) now used by ice cream makers is likewise very low in bacterial count. The ice cream mix is the only ingredient that is likely to be uncertain in this respect. If the ice cream mix has been made from high grade raw products, properly pasteurized, and processed under controlled conditions, it should contain relatively few bacteria.

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The water used in compounding the sherbet or ice mix should be of known purity and should be tested at frequent intervals, especially if the water is from a private well. Sound, fresh fruits which have been properly washed should introduce relatively few bacteria, and preserved fruits are ordinarily practically sterile. Sugar which has been kept dry contains very few bacteria. Eggs are either sterile or contain such small numbers of bacteria that the quantitative contamination from this source is negligible.

From the foregoing statements it seems logical to assume that ice or sherbet mixes, which have been made from high grade products, would contain very few bacteria before processing. If processed in clean equipment and carefully aged at low temperatures, the bacterial content of the finished product should also be relatively low. If the bacterial content of a sherbet or a water ice is high, the most likely sources of contamination are, poorly washed utensils, improper aging temperature, or, in case of a sherbet, to the addition of ice cream mix or dairy products of poor quality.

On account of the harmless nature of the ingredients used, ices and sherbets are not likely to attract the interest of the public health official. However, there would be no easier way for an inspector to detect insanitary plant methods, especially improperly washed equipment, than by means of a bacterial analysis of a water ice. The relatively low bacterial content of a water ice or sherbet enables the detection of equipment contamination of lesser magnitude than would be possible with other products, such as ice cream or milk. For example, bacterial analyses on sherbets or ices before and after freezing afford the plant manager or the inspector an excellent method of checking the efficiency of the cleaning methods in use on the freezer, and at the same time, give some measure of the general sanitary quality of the product.

The question is frequently raised as to whether or not it is necessary to pasteurize the water ice or sherbet mix. In general it may be said that the pre-heating of any food, where it is feasible, is always desirable. The high acidity of some mixes probably destroys certain pathogens, however, this factor is

neither sufficiently constant nor reliable to use as a substitute for pasteurization. On the other hand, it has been suggested that there is no more necessity for pasteurizing the water ice or sherbet mix than for similarly treating salad dressing or other such foods ordinarily prepared and consumed without previous cooking. It must be granted that even though the ingredients of water ices and sherbets are not so likely to introduce pathogenic organisms, these products are subjected to the same possibilities of human contact during processing as is the ice cream mix. Human contact with uncooked foods is always a potential source of danger and should be avoided wherever possible. Although the pasteurization of water ice and sherbet mixes may not be quite so imperative as it is for the ice cream mix, the process is

TABLE 1  
*The bacterial counts on 21 samples of orange sherbet*  
Plate count per gram of sherbet

80	2,000	20,000
180	2,100	24,000
600	3,000	31,000
700	3,400	55,000
850	3,800	64,000
1,400	7,000	180,000
1,600	15,000	1,100,000

sufficiently advantageous to more than justify its adoption. In the plants where pasteurization of the water ice and sherbet mixes is not practiced, special precautions should be taken to prevent human contact with the product and to insure the sanitary quality of the ice cream mix, dairy products, and water used.

Bacterial analyses of sherbets and ices are very seldom reported in the literature, either because of lack of interest or because it is not a common practice to make such determinations. Hammer (2) reported the analysis of 17 water sherbets which ranged from 6 to 7800 bacteria per cubic centimeter. In connection with the 1928 session of the Kansas Ice Cream Scoring Contest, as described by Fay and Martin (3), opportunity was afforded to

make bacterial analyses of 21 samples of orange sherbet. These samples were representative of the product sold in the middle west. Most of them were manufactured in Kansas, some in Missouri, and some in Nebraska.

The bacterial analyses were made on a gravimetric basis, as described by the American Dairy Science committee report on Bacteriological Methods for Examining Ice Cream (4). The difficulty encountered in expelling the air from some of the sherbets made it imperative to use the gravimetric in preference to the volumetric method. A 10-gram sample was used in order to minimize the error in weighing which resulted from the presence of relatively large pieces of fruit. Plain agar, prepared according to the standard methods of the American Public Health Association, was used, and the plates were incubated forty-eight hours at 37°C.

The results of the 21 analyses are given in table 1, and express the number of colonies per gram of sherbet.

A summary of the results shows that of the 21 samples:

10 per cent contained less than	200 bacteria per gram
24 per cent contained less than	1,000 bacteria per gram
57 per cent contained less than	5,000 bacteria per gram
76 per cent contained less than	25,000 bacteria per gram
90 per cent contained less than	100,000 bacteria per gram
10 per cent contained over	100,000 bacteria per gram

In view of the limited information at hand, it would not be feasible to suggest any definite figure as a basis for judging the bacterial content of sherbets.

The bacterial counts on these sherbets are presented solely to bring to light the possible value of bacterial analysis of sherbets and water ices as a means of detecting faulty plant methods, as well as a criterion of sanitary quality. The use of poor ingredients or improperly washed equipment is practically certain to be detected in a bacterial analysis of the finished product. The fact that a food product contains only the best grade of ingredients, and that it has been produced in clean equipment with scrupulous care is one of the most important criteria of quality. The ice cream maker who is attempting to cater to the



increasing public demand for foods of high sanitary quality may very profitably give heed to the results of bacterial analysis of his water ices and sherbets.

#### REFERENCES

- (1) FAY, A. C. 1928 Sanitary gelatin, 1914-1927. *Ice Cream Trade Jour.*, xxiv, no. 5, 57-58.
- (2) HAMMER, B. W. 1917 Bacteria in ice cream. II. Bulletin 174. Ia. Agr. Exp. Sta. Ames, Ia., p. 21.
- (3) FAY, A. C., AND W. H. MARTIN 1928 The ice cream scoring contest as a means of improving the quality of ice cream. *Jour. Dairy Sci.*, xi, no. 2, 136-142.
- (4) Committee report, 1927, Bacteriological methods of examining ice cream. American Dairy Science Assoc., *Jour. Dairy Sci.*, x, no. 5, 460-478.

## THE VOLUME OF THE CREAM LAYERS FORMING ON HOLSTEIN AND JERSEY MILK\*

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Prior to the introduction of the centrifugal cream separator the problem of the creaming ability of milk from the various breeds of cattle was of special economic importance. The numerous investigations of about twenty-five years ago compared the creaming properties of milk from the standpoint of the richness of the cream and the completeness and rapidity with which the fat in the skimmilk rose to the cream layer. These early investigations showed the milk of the Channel Island breeds to possess the best creaming properties. In recent years the fat losses in skimmilk and the test of cream obtained by gravity skimming have become insignificant but the depth of the cream layer which forms on milk is important. At the present time the old meaning of the creaming ability of milk is almost obsolete and the term will be used in this article in its newer meaning to designate the ability of milk to form a cream layer with a distinct visible line or division between the cream and skimmilk.

Recently, Palmer and Anderson (1) separated rich creams from Jersey-Guernsey and Holstein milk which were standardized with Jersey-Guernsey and Holstein skimmilk to give milks containing 3.5 per cent of fat. The Jersey-Guernsey milk gave deeper cream layers than Holstein milk which was found to be associated with the properties of the Jersey-Guernsey skimmilk rather than with the cream.

It was the intention of this investigation to determine the depth of cream layers which form upon fresh, normal milk produced by registered Holstein and Jersey cattle. The results of this study are not comparable with those in the reference just given because of differences due to standardization and aging of the products.

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The variation in the cream layer volumes caused by these factors will be considered in subsequent papers.

In view of various methods of setting milk for cream layer formation it was essential that a standard procedure should be followed for handling the milk, setting the tubes, and making the readings. A large number of tests were made using 100 cc. graduated cylinders, and test tubes as recommended by Harding (2). The results secured by the use of both containers were identical. The cylinders were more easily read, but less convenient to handle, and more expensive. For these reasons 100 cc. cylinders were used, or tubes of such a size that they could be fitted into a graduated cylinder in which position they were filled with milk and later re-inserted for reading. A slight insignificant error in the total amount of milk used in each test was introduced by the latter method.

The milk used in each test was the complete milking of one cow which was cooled to approximately 10°C. (50°F.) by a tubular surface cooler. Within a minute or two after milking and cooling, 100 cc. of each sample of milk in duplicate tubes were placed in a water bath at 2.8° to 4.4°C. (37° to 40°F.). The length of the cream layer was measured at two-, four-, and twenty-four-hour intervals. Preliminary tests showed that warm milk placed directly into tubes not in contact with other tubes was cooled to the temperature of the surrounding water in less than thirty minutes and the cream layers were comparable to those which developed on tubular cooled milk. It was also found that the temperature of the milk during the creaming period should be maintained within the specified temperatures to secure comparable results.

The samples of Holstein milk were secured from 50 registered cows maintained by L. A. Colton at a farm near the Experiment Station. The samples of Jersey milk were taken principally from the station herd of 26 cows and to a smaller extent from 22 registered Jerseys owned by C. R. Pontius of MacDougall. A total of 627 Holstein and 900 Jersey samples of milk were used in the tests. The milk was set in the milk room at the barn for cream rising observations. Records were kept of the age and health of the

cows, milk production, and stage of lactation period so that this information would be available. Most of the data were secured between June 1, 1926, and July 1, 1927.

The variations in the percentages of the fat in the milk could not be adjusted to a uniform standard without the possible introduction of some unknown variant in the creaming properties. For this reason comparable expression of results was obtained by dividing the percentage which the volume of the cream layer was of the total volume of milk by the percentage of fat which the milk contained. This result or ratio represents the percentage volume of cream for each per cent of fat which the milk contained. The majority of Holstein samples contained 3.1 to 3.7 per cent fat, whereas the Jersey milk tested from 5.0 to 7.0 per cent.

TABLE 1

*The changes in the cream layer, calculated for 1 per cent of fat, on Jersey and Holstein milk due to time held at 40°F.*

KIND OF MILK	NUM- BER OF CALVES	AVER- AGE FAT PER- CENT- AGE	2-HOUR PERIOD		4-HOUR PERIOD		24-HOUR PERIOD	
			Cream layer	No layer	Cream layer	No layer	Cream layer	No layer
Jersey.....	282	5.9	5.54	67	4.76	40	4.11	13
Holstein.....	164	3.4	5.53	24	4.85	21	4.09	14

#### INFLUENCE OF HOLDING TIME ON CREAM LAYER VOLUME

Practical experience and several investigations have shown that milk should be stored at 1.7° to 4.4°C. (35° to 40°F.) to obtain the deepest cream layer. The time of reading the depth of the cream layers has been rather variable although twenty-four hours has been most generally used. In commercial practice shorter periods of time may be more important as milk is delivered to consumers within twenty-four hours or less. The cream layers were measured after two-, four-, and twenty-four-hour periods in this study but the twenty-four-hour results were used in most calculations.

In table 1, the two-, four-, and twenty-four-hour cream layer volumes calculated for 1 per cent of fat are given for 282 samples of Jersey and 164 samples of Holstein milk. A large portion of

these samples failed to show definite cream lines within the two- and four-hour periods which was especially pronounced for Jersey milk. The volume of the cream layers formed were very similar for the milk of the two breeds, a result that will be considered again. The depth of the cream layers decreased with time but they had

TABLE 2

*The creaming ability of Holstein and Jersey milk presented on a comparable basis by calculating the normal cream layer for 1 per cent of fat*

CREAM LAYER FOR 1 PER CENT OF FAT	HOLSTEIN MILK FROM 47 COWS		JERSEY MILK FROM 47 COWS	
	Frequency	Percentage frequency	Frequency	Percentage frequency
No layer	14*	0	13*	0
2.00-2.99	8*	0	1*	0
3.00-3.19	0	0	9	3.2
3.20-3.39	5	2.5	15	5.8
3.40-3.59	9	4.8	27	9.6
3.60-3.79	22	11.9	30	10.6
3.80-3.99	28	15.3	34	12.1
4.00-4.19	35	18.9	43	15.3
4.20-4.39	36	19.4	33	11.8
4.40-4.59	26	14.1	31	11.0
4.60-4.79	16	8.6	23	8.2
4.80-4.99	8	4.3	12	4.3
5.00-5.19	0	0	7	2.5
5.20-5.39	2*		5	1.8
5.40-5.59	0		5	1.8
5.60-5.79	0		1	0.4
5.80-5.99	0		3	1.0
6.00-6.19	0		2	0.7
6.20-6.39	0		0	0
†Mean cream layer.....	4.09 ±0.02		4.11 ±0.03	
Standard deviation.....	0.55 ±0.02		0.64 ±0.02	

\* Samples not used in calculation of mean or percentage frequencies.

† The means, standard deviations, and their probable errors were calculated from the original data which yield slightly different values than those given by the data as grouped in this table.

shrunk to a minimum within the twenty-four-hour period. The shrinking of the cream layers formed on raw milk has been observed by Hammer (3) and others. It is evident that in experimental studies of creaming that observations should be made at various intervals.

## CREAMING ABILITY OF HOLSTEIN AND JERSEY MILK

The cream layer volumes which were present on Jersey and Holstein milk after twenty-four hours standing at 37° to 40°F. are summarized in table 2 and graphically represented in figure 1. The data are arranged according to frequencies of increasing cream layer volumes. The mean cream layer volume for one per cent of fat in Holstein milk was  $4.09 \pm 0.02$  and for Jersey Milk 4.11

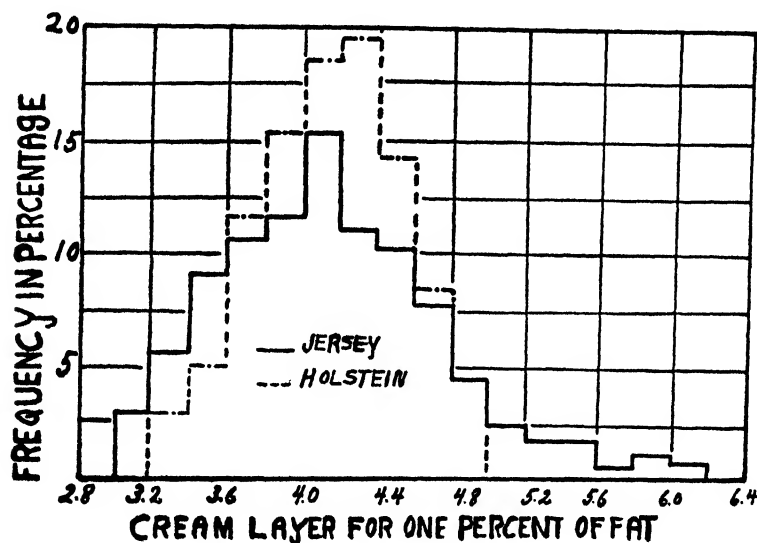


FIG. 1. THE CREAMING ABILITY OF HOLSTEIN AND JERSEY MILK SHOWN AS THE FREQUENCY, EXPRESSED IN PERCENTAGES, WITH WHICH THESE MILKS GAVE CREAM LAYERS OF VARYING VOLUMES PER 1 PER CENT OF FAT

$\pm 0.03$ . In view of the very small probable errors of these means and the slight difference between the cream layer volumes formed on these two kinds of milk it is clear that there is no difference between the cream volumes. It should be noted that the cream layer volume to be expected on any given sample of fresh Holstein or Jersey milk is rather variable since the standard deviations of the mean volumes are  $0.55 \pm 0.02$  and  $0.64 \pm 0.02$ , respectively.

These results, being contradictory to established belief, warrant

a few words concerning their accuracy. There can be no question about the similarity of the cream layer volumes for one per cent of fat which formed on either Holstein or Jersey milk used in these trials. The number of cows and samples of milk were sufficient to justify conclusions. The principal source of possible error would be that the 47 cows of each breed whose milks were used in these particular tests were not typical for the breeds due to local conditions of some character.

The milk of Jersey cows is not only more variable in the rapidity with which a cream line formed, but the final volume of the cream layer includes a wider range of percentages than does the milk of Holstein cows. Figure 1 clearly illustrates the closer grouping of the cream layer volumes of Holstein milk around the mean.

TABLE 3

*The cream layer volume, calculated for 1 per cent of fat, which formed on Holstein and Jersey milk produced in the winter and in the spring*

KIND OF MILK	WINTER			SPRING		
	Num- ber of cases	Cream layer	No layer	Num- ber of cases	Cream layer	No layer
Holstein.....	84	4.05	0	67	4.12	11
Jersey (Pontius).....				56	3.97	9
Jersey (Station).....	113	3.86	3	95	4.30	6

The standard deviations, as given in table 2, confirm the graph, for the standard deviation for the cream layer volume of Jersey milk is 0.09 greater than that of Holstein milk.

It was recognized at the beginning of this study that factors other than the breed of cattle may influence the normal creaming ability of the milk as it is drawn from the cow. Records were kept of the age and health of the cow, regularity of milking periods, amount of milk given and stage of the lactation period. In a few isolated cases the results obtained with certain samples of milk were discarded due to these causes since the samples were taken with a knowledge of the possibility of discrepancies due to them. The data were grouped on the basis of the season of the year in which the milk was produced so that the creaming ability

of milk produced in the winter on dry feeds, including silage and mangels, could be compared with the creaming properties of milk produced on pasture grass. The summarized figures for Jersey and Holstein milk produced under these conditions are given in table 3.

TABLE 4

*The creaming ability of milk from individual cows presented on a comparable basis by calculating the normal cream layer for 1 per cent of fat*

COW NUMBER	HOLSTEIN HERD					JERSEY HERD (PONTIUS)					JERSEY HERD (STATION)				
	Number of trials	Cream layer for 1 per cent of fat				Number of trials	Cream layer for 1 per cent of fat				Number of trials	Cream layer for 1 per cent of fat			
		Min-imum	Max-imum	Aver-age	No layer		Min-imum	Max-imum	Aver-age	No layer		Min-imum	Max-imum	Aver-age	No layer
1	6	3.3	3.9	3.6	0	4	3.7	5.8	4.5	0	13	3.3	4.8	4.0	0
2	4	4.1	4.5	4.3	0	4	3.3	4.6	3.8	0	13	3.3	4.9	4.1	0
3	6	4.0	4.7	4.3	0	4	3.7	5.6	4.6	0	11	3.8	5.4	4.1	0
4	5	3.7	4.7	4.0	0	4	4.3	5.5	4.9	2	13	3.4	4.6	3.9	0
5	6	3.5	4.1	3.9	0	3	3.3	5.0	3.9	0	14	3.8	6.1	4.7	6
6	6	3.7	4.6	4.1	0	4	3.5	5.7	4.3	0	10	3.2	6.8	5.2	2
7	4	4.3	4.5	4.4	0	3	4.4	6.1	5.1	0	11	3.9	4.9	4.2	0
8	6	4.0	4.8	4.3	0	3	4.6	4.6	4.6	2	9	4.3	5.3	4.8	0
9	6	3.6	4.3	3.9	0	4	3.9	4.7	4.3	0	13	3.1	4.5	3.9	0
10	4	3.7	4.3	4.0	0	4	3.5	5.5	4.3	0	13	3.5	4.8	4.1	0
11	4	3.5	3.8	3.6	0	4	3.9	4.8	4.2	1	12	3.3	4.6	4.0	0
12	6	4.0	5.2	4.5	0	4	3.4	5.0	4.1	0	9	3.0	4.5	3.7	0
13	3	4.0	4.3	4.2	0	4	3.8	5.4	4.5	0	7	4.7	6.0	5.4	1
14	3	3.4	3.9	3.7	0	4	4.3	5.6	4.9	0	10	4.1	6.1	4.7	0
15	3	4.4	4.5	4.5	0	4	3.0	4.8	3.9	0	7	3.6	6.2	4.9	0
16	5	3.8	4.6	4.2	0	4	3.9	4.7	4.3	0	9	3.2	4.5	3.7	0
17	5	4.0	4.8	4.3	0	3	4.1	5.6	4.7	0	10	3.3	4.7	3.9	1
18	3	3.5	4.0	3.8	0	4	3.5	4.4	3.9	0	8	3.6	4.1	3.7	1
19	3	3.5	3.8	3.7	0	4	3.3	5.0	4.1	0	8	3.5	4.9	4.0	0
20	3	4.3	4.4	4.3	0	2	3.2	3.4	3.3	0	3	3.1	3.8	5.5	0

The grouping of the figures in this manner again illustrates the uniform mean cream layers, calculated for 1 per cent of fat in the milk, which form on Holstein and Jersey milk. The more uniform creaming of Holstein milk is also shown. There was no significant difference between the creaming properties of Holstein milk produced in the spring and winter months. The interpreta-



tion of the results secured with Jersey milk is difficult because there appears to be a difference between the creaming ability of milk produced by the station Jersey herd which is contradicted of the creaming properties of the spring milk produced by the

TABLE 5

*The relationship of cream layer, per 1 per cent of fat, to specific gravity of milk*

SPECIFIC GRAVITY OF MILK	PER CENT FAT	TOTAL SOLIDS CALCULATED	CREAM LAYER		
			2-hour period	4-hour period	24-hour period
1.0290	4.2	12.3	No layer	5.2	4.8
1.0300	5.0	13.5	7.4	5.4	4.2
1.0301	5.4	14.0	No layer	5.2	4.4
1.0303	5.5	14.2	5.1	3.2	2.9
1.0309	5.4	14.2	No layer	No layer	No layer
1.0313	5.7	14.6	No layer	4.9	4.2
1.0325	2.8	11.5	5.3	4.6	4.3
1.0325	5.6	14.8	5.4	5.0	3.9
1.0327	2.9	11.6	5.5	4.9	4.4
1.0327	5.0	14.2	No layer	7.2	5.2
1.0330	2.8	11.5	4.6	4.6	4.3
1.0331	6.0	15.5	No layer	5.1	4.3
1.0333	6.6	16.2	No layer	5.0	4.2
1.0335	6.2	15.8	No layer	4.5	4.0
1.0335	6.2	15.8	No layer	No layer	No layer
1.0339	4.8	14.2	5.2	5.0	4.2
1.0339	5.6	15.2	No layer	6.4	5.0
1.0340	4.0	13.3	6.2	5.2	4.7
1.0340	6.2	15.9	No layer	5.4	4.6
1.0340	6.5	16.3	No layer	No layer	No layer
1.0342	5.3	14.9	No layer	6.8	5.2
1.0343	4.7	14.3	5.9	5.7	4.7
1.0350	4.5	14.1	6.2	4.9	4.0
1.0350	4.8	14.5	No layer	4.6	3.9
1.0350	4.8	14.5	5.0	4.6	3.7
1.0350	4.8	14.5	5.4	5.0	3.7
1.0366	4.2	14.1	6.2	5.2	5.0
1.0370	4.2	14.3	6.2	5.7	5.2

other Jersey herd. Considered as a whole, these cream layers which formed on 415 samples of milk are not sufficient in number to show a difference between the creaming ability of milk produced in the winter or spring.

These data were also arranged and studied to show a relationship between the fat content of milk and the cream layer volume per 1 per cent of fat. When the milks of both breeds were considered together there were sufficient samples testing between 4 and 5 per cent to give a complete range of tests from 3 to 7 per cent. There appeared to be no other relationship between the percentage of fat and the cream layer volume than the value already given of 4.1 per cent of cream layer for each 1 per cent of fat in the milk. The data are not presented according to richness of the milk since no difference in the cream layer volumes per 1 per cent of fat were observed in table 2 comparing Holstein and Jersey milk.

A number of specific gravity determinations were made on samples of normal milk by the Westphal Balance. The results of these determinations, together with the cream layers which formed on the milk after two, four, and twenty-four hours, are given in table 5. The specific gravity of the milk was not associated with the creaming ability of the milk, a conclusion which is in agreement with the work of Palmer, Hening, and Anderson (4), and others.

#### VARIATIONS IN CREAMING ABILITY OF MILK FROM INDIVIDUAL COWS

Hammer (3) found that the cream layer volumes for milk produced by 10 different cows varied from 4.9 to 7.4 for each 1 per cent of fat contained in the milk. Others have observed this same variation. The cream layer measurements made in this investigation permitted a more extensive study of this factor as a variant in the creaming ability of milk.

The data given in table 4 include cream layer measurements made from 2 to 14 milk samples taken from each cow. The milk from 60 cows was used in this comparison. The cows varied in age, production, and stage of lactation period. Not only does the creaming of the milk from one cow differ from that of another but in most instances the different samples of milk from the same cow vary widely. In a few cases a cow produced milk which, from day to day, possessed rather uniform creaming powers.

Expressed in another way, this study has shown that one cannot predict with accuracy the creaming ability of the milk of an individual cow from a knowledge of previous cream layer determinations on the milk. An exception must be made for cows that produced milk which gave no visible cream line in a large percentage of trials and for certain cows which gave milk that tended to give abnormally deep or shallow cream layers even though their depth varied from sample to sample.

#### SUMMARY

1. The comparative creaming properties of milk may vary according to the procedure followed in making the tests. For greatest uniformity and the exclusion of unknown factors the milk was set for creaming within a few minutes after milking in ice water at 2.8° to 4.4°C. The cream layers of different samples showed variations in the cream layer volume and the distinctness of the cream line after two or four hours which were less pronounced after twenty-four hours.

2. The mean depth of the cream layers forming on normal milk from Holstein and Jersey cows was directly proportional to the percentages of fat which they contained. The percentage which the cream layer represented of the total volume of milk was about 4.1 times the percentage of fat.

3. The variations in the cream layer volumes forming on individual samples of Jersey milk were greater than those for Holstein milk. This difference was especially noticeable after two and four hours of creaming.

4. The milk from an individual cow did not possess uniform creaming properties from milking to milking although there was a tendency for the milk from certain cows to give either large or small cream layer volumes or a high percentage of no layer cases.

5. The cream layer volumes on the samples of milk used in these tests were not affected by the season of the year in which the milk was produced. The milk from Holstein cows remained most uniform throughout the year.

6. Variations in specific gravity of milk were not related to changes in creaming properties.

## REFERENCES

- (1) PALMER, L. S., AND ANDERSON, E. O.: Physico-chemical factors influencing cream rising. I. Viscosity. *Jour. Dairy Sc.*, 1926, ix, 1-14.
- (2) HARDING, H. A.: The measurement of the volume of cream on milk. *University of Illinois Circ. No. 249*, 1921.
- (3) HAMMER, B. W.: Studies on the creaming ability of milk. *Iowa State College, Research Bul.*, No. 31, 1916.
- (4) PALMER, L. S., HENING, J. C., AND ANDERSON, E. O.: Physico-chemical factors influencing cream rising. II. Relation of plasma colloids to pasteurization effects. *Jour. Dairy Sc.*, 1926, ix, 171-191.

## THE CONCENTRATED WATER SOLUBLE FRACTION OF MILK AS A SOURCE OF VITAMIN B\*

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Although milk as a source of the vitamin B complex is not particularly noteworthy, its quota of this accessory food factor is sufficiently adequate to warrant the assumption that a concentrate of the water soluble fraction would contain an appreciably greater quantity of this vitamin than is found in the natural milk or in any of its concentrated commercial forms.

Such a concentrate has been prepared under commercial and semi-commercial conditions. Briefly, the procedure involves the successive removal from the milk, of the fat or cream by skimming, the casein by heating with a suitable precipitant, the lactalbumin by coagulating with heat, a considerable proportion of the insoluble calcium phosphate, and by repeated crystallizations, the greater part of the milk sugar. The residual liquor or serum may be concentrated to any desired degree, as for example to a viscous fluid, containing about 40 per cent solids, a thick paste containing 65 to 80 per cent solids, or even to dryness. Since the method of preparation is carried out with the object of utilizing the obtainable water soluble constituents of milk, minus those minor increments lost by adsorption and occlusion and minus a large part of the lactose, the final serum or liquor contains the so-called soluble nitrogen extractives, the water soluble milk minerals, a certain proportion of milk sugar and the vitamin B of the original fluid milk, concentrated to a high degree.

The composition of the dry solids of the water soluble fraction, prepared as described, in comparison with the composition of the dry solids of milk is shown in table 1. The alteration of the mineral balance is shown by comparing the ash constituents of natural milk with those of the water soluble fraction as in table 2.

\* Received for publication June 19, 1928.

TABLE 1

*The composition of milk solids and the solids of the water soluble fraction*

	MILK SOLIDS (DRY BASIS)	COMMERCIALY PREPARED WATER SOLUBLE FRACTION (DRY BASIS)
	per cent	per cent
Fat.....	27.25	00.00
Ash.....	6.25	28.37
Lactose.....	39.00	57.27
Total nitrogen.....	4.31	2.15
Protein nitrogen.....	4.04	0.67
Non-protein nitrogen.....	0.27	1.48
Ammonia nitrogen.....		0.19

TABLE 2

*The composition of the ash constituents of milk solids and the solids of the water soluble fraction*

	MILK SOLIDS (ASH CONSTITUENTS)	COMMERCIALY PREPARED WATER SOLUBLE FRACTION (ASH CONSTITUENTS)
	per cent	per cent
P <sub>2</sub> O <sub>5</sub> .....	26.87	4.28
CaO.....	22.28	8.31
MgO.....	2.48	2.11
K <sub>2</sub> O.....	27.83	34.89
Na <sub>2</sub> O.....	6.64	15.27
Cl.....	13.28	41.08
SO <sub>3</sub> .....	2.25	1.44
Undetermined.....	2.02	2.10
Total.....	103.65	109.48
0 = Cl.....	3.65	9.48
	100.00	100.00

#### VITAMIN B CONTENT OF THE SOLIDS OF THE WATER SOLUBLE FRACTION

Since the particular object of this study was to determine the relative vitamin B content of the solids of the water soluble fraction of milk, prepared as already indicated, numerous feeding experiments have been conducted in parallel with variable quanti-



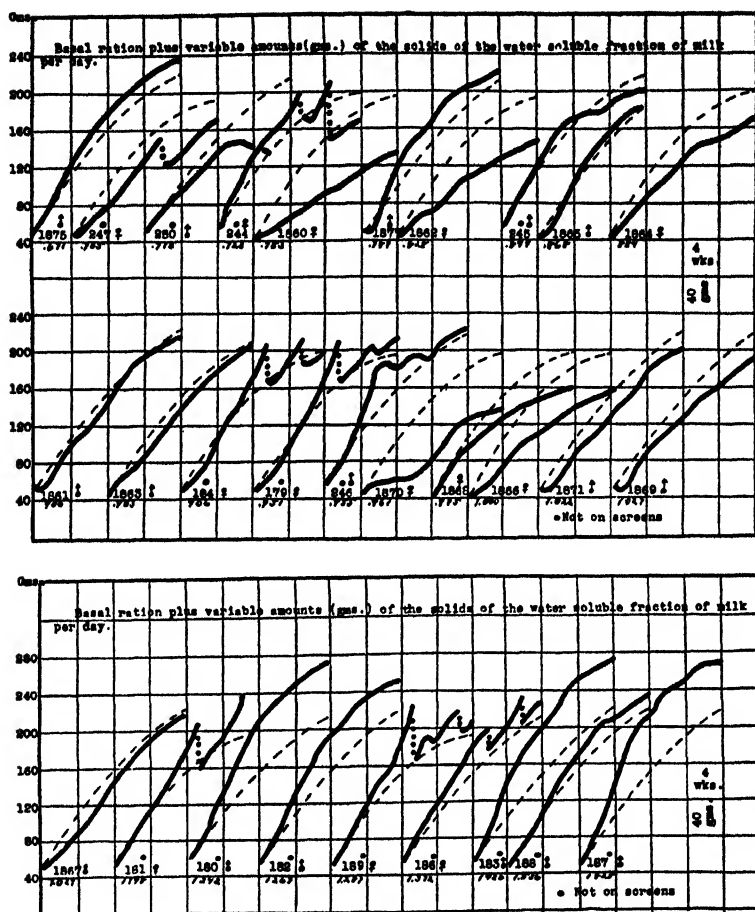


CHART 2 (continued). The SOLIDS OF THE WATER SOLUBLE FRACTION OF MILK AS A SOURCE OF VITAMIN B

ties of dry yeast. White rats reared under standardized conditions at our own laboratory were used. At the age of twenty-eight to thirty days, young animals weighing from 40 to 50 grams were placed in individual cages with screened bottoms, (see exceptions noted on accompanying charts). The following basal ration was fed: casein purified by acetic acid, 18 parts; salt



mixture no. 40,<sup>1</sup> 4 parts; powdered agar agar, 2 parts; butterfat, 5 parts; dextrin, 71 per cent. Supplementing the basal ration, variable quantities of the solids of the water soluble fraction of milk, or dried yeast were fed daily as the sole source of vitamin B. Dried yeast was selected as the basis of comparison, because of its widely known vitamin B content and because of its general use as a carrier of this important accessory food factor. The yeast used for these experiments was fresh brewers' yeast obtained direct from the manufacturers, immediately dried and ground at our own laboratory. For those animals not kept in screened bottom cages, the yeast and milk concentrate were mixed with the basal ration; for all others the designated amounts were fed separate in the form of a thin paste or pellet.

The accompanying growth curves (charts 1 and 2) adequately illustrate the comparative vitamin B potency of dried yeast and the solids of the water soluble fraction of milk with composition and method of preparation as already stated. The evidence is fairly consistent in indicating a vitamin B content of the milk concentrate substantially equivalent to that of dried yeast. Figures 1 to 5 on plate 1 show the effect of variable amounts of the solids of the water soluble fraction of milk as the sole source of vitamin B. These animals were photographed at the age of eleven weeks, or seven weeks after continuous feeding of the experimental ration. Complete adequacy of vitamin B is illustrated by the condition of the animal in figure 1. This animal received 8.7 per cent of the solids of the milk concentrate mixed with the basal ration. During the first seven weeks of the experimental feeding period the average daily intake of the milk concentrate solids was 1.019 grams. Lesser amounts were furnished the animals shown in figures 2, 3 and 4, the average daily intake amounting to 0.359, 0.063 and 0.067 gram respectively. The animal shown in figure 5 received no vitamin B from any source whatsoever, and typifies the results of such complete deficiency.

<sup>1</sup> Steenbock, H., and Nelson, E. M., *Jour. Biol. Chem.*, lvi, p. 362, 1923.

## CONCLUSIONS

Data are given which show that the solids of the water soluble fraction of milk, when properly prepared and concentrated, are highly potent in vitamin B content and compare favorably with dried yeast as a source of the growth promoting and antineuritic factor.

## PLATE 1

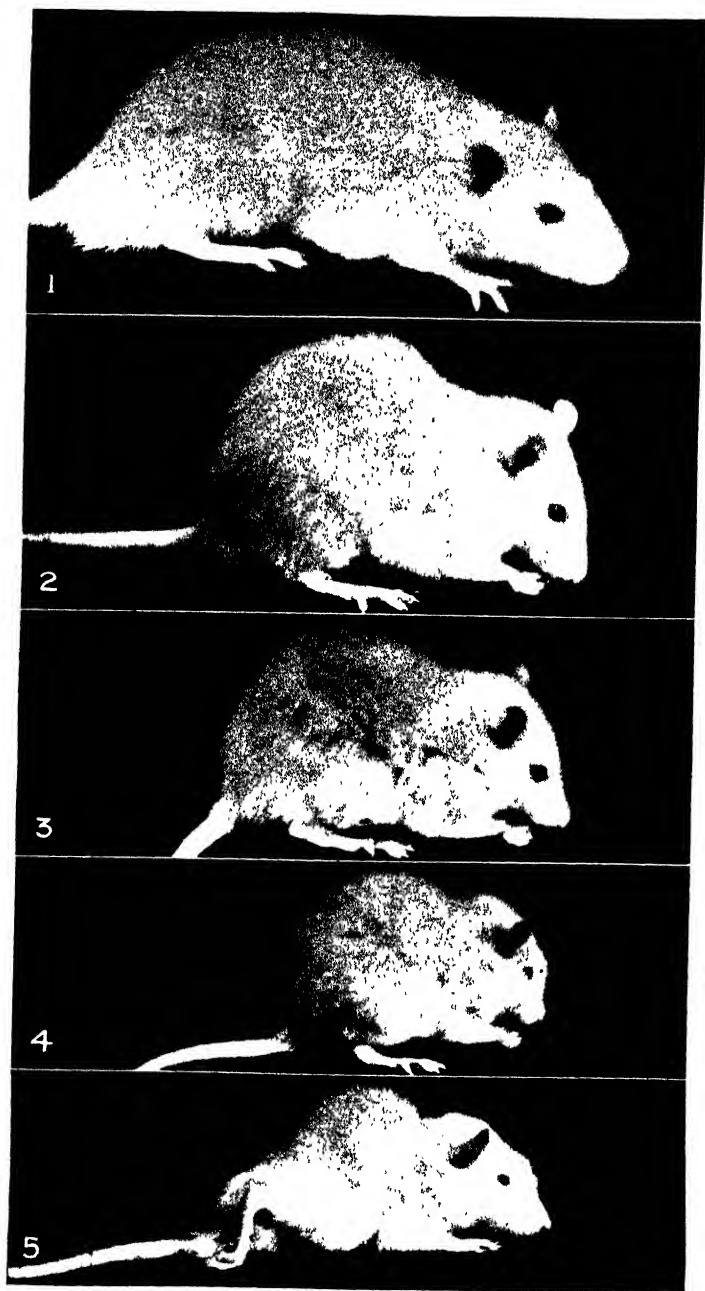
FIG. 1. Results obtained from feeding 1.019 grams daily for seven weeks of the solids of the concentrated water soluble fraction of milk as the sole source of vitamin B.

FIG. 2. Results obtained from feeding 0.359 gram daily for seven weeks of the solids of the concentrated water soluble fraction of milk as the sole source of vitamin B.

FIG. 3. Results obtained from feeding 0.063 gram daily for seven weeks of the solids of the concentrated water soluble fraction of milk as the sole source of vitamin B.

FIG. 4. Results obtained from feeding 0.067 gram daily for seven weeks of the solids of the concentrated water soluble fraction of milk as the sole source of vitamin B.

FIG. 5. Results obtained from feeding basal ration only without any vitamin B whatsoever for seven weeks.





# THE EFFECT OF LECITHIN IN DAIRY PRODUCTS UPON BUTTER FAT DETERMINATIONS\*

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## INTRODUCTION

The loss of butter fat in dairy manufacturing processes, such as churning, was considered to be relatively unimportant when tests for the fat content were made by the regular Babcock test. But since the introduction of the butyl alcohol modification of the Babcock test, and the wider use of ether extraction methods, the fat losses appear to be considerably greater than was formerly supposed. Tested by the older method, the fat content of buttermilk showed tests of about 0.2 per cent. Tests recently made on a large number of samples from various creameries have shown that the average fat content of the buttermilk from Iowa creameries is 0.7 per cent. The question now arises, which method, if either, gives the true percentage of fat?

## LITERATURE REVIEW

Thurston (12) has indicated that fat is not the only constituent of milk which is indicated by the fat tests, but that the results are influenced by the presence of lecithin. This appears to be quite probable, because of the similarity of lecithin to fat in its properties, such as its solubility. Whether or not the lecithin occurs in large enough amounts to cause an appreciable error in fat tests can be determined by comparing the amounts of lecithin and of fat in various dairy products.

That milk contains no lecithin is the belief of Schlossman (10), who reports that the phosphorus compounds which are generally believed to be lecithin, are in reality decomposition products of casein. This view is not held by other investigators, who have shown that lecithin is present in milk, and have determined the

\* Received for publication June 9, 1928.

amount which is present. The results of some of these determinations made on cow's milk are given in table 1.

These results indicate that the average amount of lecithin present in milk is about 0.075 per cent. If we consider milk as having an average fat content of 3.6 per cent, then the error in the fat determination due to the presence of lecithin would be about two per cent, provided that the entire amount of lecithin figured in the determination.

While most of the results reported are for milk, some determinations have been made on the amount of lecithin in other products. Bordas and de Raczkowski (2) found 0.334 per cent in cream containing 50.88 per cent fatty material. Dornic and Daire (5) report values of 0.0905 and 0.0944 per cent for raw

TABLE 1  
*Lecithin content of cow's milk as found by various investigators*

AUTHOR	PER CENT LECITHIN	AVERAGE VALUES
Stoklassa (11).....	0.09 -0.113	0.1015
Burow (3).....	0.049 -0.1058	0.0535
Koch and Woods (7).....	0.072 -0.086	0.0797
Nerking and Haensel (9).....	0.0364-0.1163	0.0629
Glikin (6).....	0.0158-0.1173	0.0765

cream and of 0.0651 for pasteurized cream. While it is evident from these results that the lecithin content of cream is higher than that of milk, the percentage error in the fat determinations due to the presence of lecithin is of course considerably less because of the high fat content of the cream. Cusick (4) found the lecithin content of butter to vary between 0.0433 and 0.0723 per cent. Since the fat content of butter is ordinarily greater than 80 per cent, the lecithin would exert practically no influence on the determination.

Because skimmed milk and buttermilk contain much less fat than milk or cream, small amounts of lecithin in these substances will cause a greater error in the fat determinations than in milk, cream or butter. But few determinations of lecithin have been reported on skimmed milk and buttermilk. Of these, Dornic

and Daire (5) found 0.0332 per cent of lecithin in buttermilk which contained 0.42 per cent of fatty material. Bischoff (1) found that the phosphatides remain for the most part in the buttermilk. Bordas and de Raczowski (2) report 0.018 per cent in skimmed milk which contained 0.09 per cent of fatty material. The error due to lecithin in the fat determination would thus be about 8 per cent for buttermilk, and 20 per cent for skimmed milk.

The results obtained by these investigators indicate that the presence of lecithin in substances of low fat content will exert considerable influence upon the results of the determination of the fat content.

TABLE 2  
*Lecithin in milk products*

MATERIAL TESTED	PER CENT OF FATTY MATTER	PER CENT OF LECITHIN			PER CENT OF THE EXTRACT THAT IS LECITHIN
	Average	Low	High	Average	
Milk.....	3.848	0.0345	0.0709	0.0447	1.6
Cream.....	45.70	0.1824	0.2155	0.1981	0.43
Skimmed milk.....	0.153	0.0082	0.0290	0.0165	10.78
Buttermilk.....	0.643	0.1036	0.1480	0.1302	20.25

#### EXPERIMENTAL WORK

##### *The amount of lecithin in dairy products*

The experimental work has consisted of two parts: First, the determination of the amount of phosphatides, calculated as distearyl lecithin, present in the ether extract of various dairy products, the extraction having been made by the Mojonner modification of Röse-Gottlieb method. Second, the preparation of lecithin, and its addition to buttermilk in varying amounts, followed by the determination of fatty matter.

To determine lecithin, samples of milk or cream or other material were extracted in the regular manner. A number of these extracts were combined to give a sufficiently large sample. The ethers were then evaporated, the residue dried and weighed,



then taken up with anhydrous ether or chloroform, transferred to a platinum dish, evaporated to dryness, and then fused with  $K_2CO_3$  and  $NaNO_3$ . The phosphorus was then precipitated with ammonium molybdate, then as  $MgNH_4PO_4$ , and finally converted to  $Mg_2P_2O_7$ . From this lecithin may be calculated by using the factor 7.27, considering the lecithin to be of the di-stearyl type. The results thus obtained represent the amount of lecithin in the ether extract, but not necessarily the total amount of lecithin present in the original material.

A summary of results is given in table 2.

The results show that the lecithin is extracted in amounts great enough to cause a considerable error in the fat determination of skimmed milk and buttermilk. For skimmed milk this error would be 10.78 per cent, and for butter milk, 20.25 per cent.

#### *The effect of added lecithin on fat determinations*

Lecithin was prepared from the yolks of eggs according to the method of MacLean (8). The results of the analysis of this material as prepared were, phosphorous 4.03 per cent and nitrogen 1.84 per cent. The values given by MacLean are, phosphorous 4.0 per cent and nitrogen 1.8 per cent.

Weighed amounts of lecithin were then added to buttermilk, and fat determinations made on the buttermilk alone, and on the buttermilk after the addition of the lecithin. Three methods of testing were used: (a) Babcock, using skim milk test bottles, (b) Butyl alcohol modification of the Babcock, also using skim milk bottles, and (c) the Mojonnier test. The difference in the tests divided by the amount of lecithin added to 100 cc. of butter milk and multiplied by 100 gives the percentage recovery of lecithin.

The results of these tests are summarized in tables 3, 4 and 5. The data show that the results of the fat tests are increased by the presence of lecithin, although all of the lecithin is not recovered by the tests. In the Babcock test, 71 per cent of the lecithin added affects the result, in the butyl alcohol modification, 68.5 per cent, and in the Mojonnier test, 76.5 per cent. The last result is lower than expected, in view of Glikin's (6) work.

TABLE 3

*The effect of added lecithin on fat tests made by the Babcock method*

WEIGHT OF ADDED LECITHIN IN 100 CC.	TEST BEFORE ADDITION OF LECITHIN	TEST AFTER ADDITION OF LECITHIN	DIFFERENCE	PER CENT OF ADDED LECITHIN WHICH IS RECOVERED
<i>grams</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
0.1046	0.075	0.09	0.015	14.3
0.1484	0.25	0.33	0.08	53.9
0.0540	0.105	0.19	0.085	157.4
0.1808	0.13	0.19	0.06	33.2
0.1035	0.11	0.22	0.11	106.3
0.1063	0.11	0.23	0.12	113.0
0.1044	0.10	0.175	0.075	71.8
0.2083	0.10	0.20	0.10	48.0
0.1075	0.10	0.15	0.05	46.5
0.2005	0.10	0.23	0.13	64.8
0.1526	0.09	0.20	0.11	72.1
Average.....				71.0

TABLE 4

*The effect of added lecithin on fat tests made by the butyl alcohol method*

WEIGHT OF ADDED LECITHIN IN 100 CC.	TEST BEFORE ADDITION OF LECITHIN	TEST AFTER ADDITION OF LECITHIN	DIFFERENCE	PER CENT OF ADDED LECITHIN WHICH IS RECOVERED
<i>grams</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
0.1088	0.47	0.578	0.108	99.2
0.1046	0.46	0.525	0.065	62.1
0.1484	0.65	0.78	0.13	87.6
0.2122	0.55	0.65	0.10	47.1
0.0540	0.37	0.41	0.04	74.0
0.0618	0.42	0.43	0.01	16.2
0.1808	0.60	0.75	0.15	83.0
0.1035	0.56	0.59	0.03	29.0
0.1044	0.47	0.535	0.065	62.3
0.2083	0.47	0.69	0.22	105.6
0.1075	0.46	0.50	0.04	37.2
0.2005	0.46	0.65	0.19	94.7
0.1510	0.48	0.61	0.13	86.1
0.2510	0.48	0.67	0.19	75.7
0.1526	0.48	0.58	0.10	65.5
0.2550	0.48	0.66	0.18	70.6
Average.....				68.5

It is evident then that the results of the determinations of the amount of lecithin present in milk products, as determined on the ether extracts, are too low, since only 76.5 per cent of the lecithin is recovered. When this correction is made, the average lecithin content of butter milk becomes 0.1702 per cent, rather than 0.1302 per cent. The 0.1302 per cent, however, represents

TABLE 5  
*The effect of added lecithin on fat tests made by the Mojonnier method*

WEIGHT OF ADDED LECITHIN IN 100 CC.	TEST BEFORE ADDITION OF LECITHIN	TEST AFTER ADDITION OF LECITHIN	DIFFERENCE	PER CENT OF ADDED LECITHIN WHICH IS RECOVERED
<i>grams</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
0.1484	0.683	0.821	0.138	93.0
0.2122	0.580	0.762	0.182	85.7
0.0540	0.482	0.517	0.035	64.8
0.0618	0.488	0.543	0.055	89.0
0.1808	0.613	0.726	0.113	62.5
0.1000	0.612	0.694	0.082	82.0
0.1035	0.611	0.662	0.051	49.3
0.1063	0.611	0.666	0.055	51.7
0.1044	0.551	0.643	0.092	88.1
0.2083	0.551	0.729	0.178	85.5
0.1075	0.551	0.637	0.086	80.0
0.2005	0.551	0.723	0.172	85.7
0.1510	0.559	0.654	0.095	62.8
0.2510	0.559	0.752	0.193	76.9
0.1526	0.559	0.684	0.125	81.9
0.2550	0.559	0.775	0.216	84.7
Average.....				76.5

the amount which is removed with the fat. Then the average fat content of the buttermilk from Iowa creameries is 0.57 per cent, rather than 0.7 per cent.

#### SUMMARY AND CONCLUSIONS

Previous investigators have shown that milk and milk products contain phosphatides, and suggested that these substances might influence the results of fat determinations.

The work reported in this paper shows that skimmed milk

and buttermilk contain phosphatides in amounts large enough to give results in the determination of fat which are appreciably high. It also shows that lecithin, the principal phosphatide present in milk, when added to buttermilk, causes a high result. The increase is approximately the same for the three methods of testing which were used.

The work also indicates that the average fat loss in buttermilk is 0.57 per cent, rather than 0.7 per cent, the difference being due to the amount of lecithin which appears in the fat test.

#### REFERENCES

- (1) BISCHOFF, GEORGE: Zur Kenntnis der Milch vorhandenen Phosphatide. *Zeit. physiol. Chem.*, 1928, clxxiii, 227-32.
- (2) BORDAS, F., AND DE RACZKOWSKI: Variation de l'acide phosphorique suivant l'âge de lait. *Comp. rend. Acad. Sci.*, 1902, cxxxv, 302-303.
- (3) BUROW, R.: Der Lecithingehalt der Milch und seine Abhängigkeit von relativem Hirngewichte des Säuglings. *Zeit. physiol. Chem.*, 1900, xxx, 495-507.
- (4) CUSICK, J. T.: Reported by SUPPLEE, G. C.: The lecithin content of butter and its possible relationship to the fishy flavor. *Cornell Univ. Agr. Exp. Sta., Memoir 29*, p. 110, 1919.
- (5) DORNIC AND DAIRE: Contribution a l'étude de la Lecithine du lait. *Ann. Fals.*, 1910, xxx, 533-538.
- (6) GLIKIN, W.: Zur Biologischen Bedeutung des Lecithens. III. Über den Lecithin and Eisengehalt in der Kuh- und Frauenmilch. *Biochem. Zeit.*, 1909, xxi, 348-354.
- (7) KOCH, W., AND WOODS, H. S.: The quantitative estimation of the lecithins. *Jour. Biol. Chem.*, 1905, i, 203-210.
- (8) MACLEAN, H.: Lecithin and allied substances. The lipins. *Monographs on Biochemistry 78-81*. Longmans, Green & Co., London, 1918.
- (9) NERKING, J., AND HAENSEL, E.: Der Lecithingehalt der Milch. *Biochem. Zeit.*, 1908, xiii, 348-353.
- (10) SCHLOSSMANN: Reported by DORNIC AND DAIRE: *Ann. Fals.*, 1910, xxx, 533-538.
- (11) STOKLASSA, J.: Zur Kenntnis des Phosphors in der Frauen- und Kuhmilch. *Zeit. Physiol. Chem.*, 1897, xxiii, 343-346.
- (12) THURSTON, L. M.: Address before National Creamery Buttermakers Association, 1927.

# THE EFFECT OF DISTILLED WATER UPON THE TENDENCY TO COLONY FORMATION UPON PETRI PLATES\*

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The macroscopic colony count has been used for many years as a measure of the bacterial population of milk. In the numerous laboratories throughout the country it is considered the standard method, supported as it is, by the American Public Health Association. The first edition of Standard Methods mentioned only the plate method, which seemed to be adequate for the kinds of milk produced at that time. The numbers of bacteria found in milk were high as a rule, and one had but to separate the bad from the worse. In the four subsequent editions of Standard Methods other methods have claimed attention and obtained supporters, somewhat to the detriment of the plate method. Paralleling this diversity of methods there likewise has been an improvement in the milk itself, for there is no longer the large spread between milk of high and low quality. Such conditions place a greater tax upon the method which was devised primarily to eliminate the poorest milk.

There is small wonder then that the plate method has seemed to be less accurate. Too much is expected of it today. It is not an instrument of precision, which fact is recognized by the revising committees, as is evidenced by the statement in the introduction to the various editions of Standard Methods. Furthermore, it has recently been shown that Standard Methods have not had a fair chance. The Report of the Referee for the Bacteriological Examination of Milk (1) indicates that many brands of peptone and beef extract are used in amounts varying as much as 100 per cent from those considered standard. The reaction of the medium, the time and temperature of incubation were frequently quite different from those permitted. Now the methods have

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been subjected to repeated criticism, which seem on thoughtful consideration, to be unwarranted. Thus, one would be inclined to think that much of this criticism would tend to disappear were Standard Methods adhered to a little more carefully. It is the purpose of this paper to show that this is true and that one factor has been overlooked in the previous discussions. Most of the criticism has been that there was little constancy in the method; that duplicate plates poured at the same time would vary to a great degree. The work of Wright and Thornton (2) tended to show that this criticism was justifiable because they carefully made large numbers of plates from the same dilution, taking every precaution that all variables were controlled. They report that variations existed of such magnitude that duplicate values were hardly to be expected. They back their contention with mathematical proof, since they subjected their results to the usual statistical analysis. In biological data, however, it is questionable whether implicit faith can be placed in such proof, for many factors enter into the simplest of biological phenomena, some of which are known, some are not. If, in addition to the known variables, one takes into consideration the fact that variables also exist which are either imperfectly known or are even unknown, then the mathematical interpretation is to a certain extent, invalidated. Wright and Thornton's paper seemed so convincing that their work was repeated by ourselves (3) with rather interesting results.

A single dilution of milk was prepared of such a bacteria content, as determined by the direct count, that plates would have 150 to 200 colonies. From this dilution 75 plates were poured, 15 at a time, numbering each plate consecutively as it was poured. After incubation and counting statistical analyses were made from which the usual constants were obtained. We found great variations, as did Wright and Thornton, but by numbering the plates, a fact was disclosed which led to still further work. It was ascertained that for the first few plates poured the count remained quite constant, but after this the colony count increased regularly. Figure 1 is a scatter diagram on one of these experiments, showing the colony count for each of the 75 plates as they were poured.

If each point on this graph were joined, a saw-toothed curve would be the result. The saw-teeth would represent the minor variations, which are doubtlessly inherent in the method, but superimposed upon these is a major trend, which certainly cannot be ascribed to the usual minor variations mentioned above.

Figure 2 is derived from the same data as the previous figure, but the running averages of 5 counts are plotted instead of

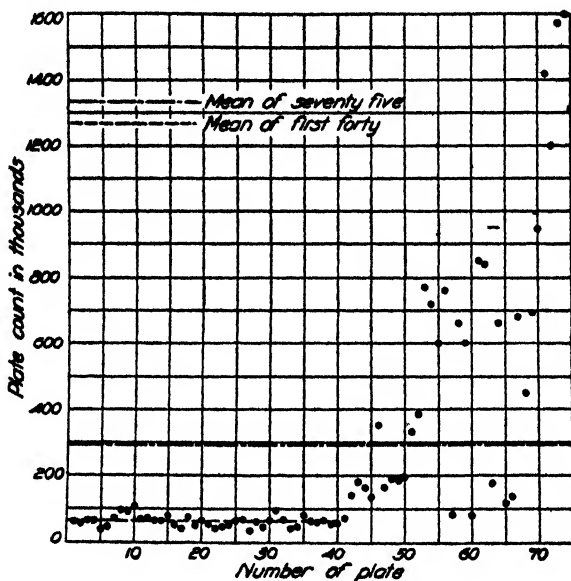


FIG. 1. DISTRIBUTION OF BACTERIA COUNTS THROUGH 75 PLATES SHOWING THE TENDENCY FOR THE COLONY COUNT TO INCREASE AS TIME INCREASES

each individual count. This overcomes the effect of the minor variations.

Figures 3, 4 and 5 are also given to show the constancy with which the increase is noted. Through these 5 plate averages a smooth curve can be drawn with very few non-conforming points. This curve indicates in a much better way than does figure 1 the increase in colony count which takes place when the dilution water is allowed to stand. At first thought, one would be tempted to ascribe this upward trend to growth, since the curve

itself is quite similar to a typical growth curve, but it should be pointed out at this time that this upward trend has been found in

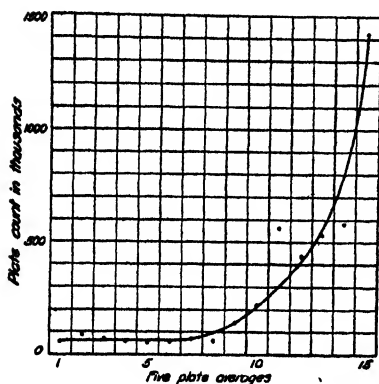


FIG. 2

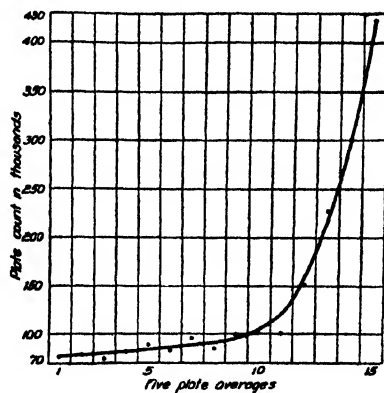


FIG. 3

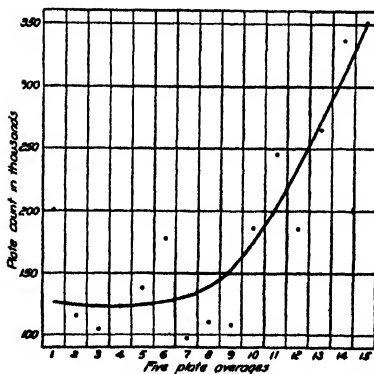


FIG. 4

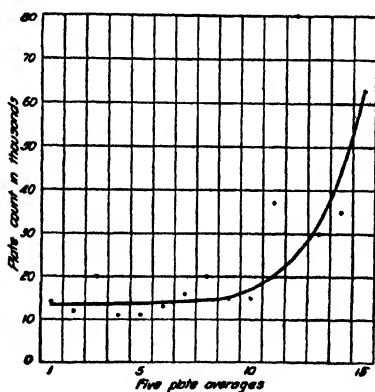


FIG. 5

FIG. 2. CURVE OBTAINED FROM PLOTTING FIVE PLATE AVERAGES OF COUNTS SHOWN IN FIGURE 1

FIGS. 3, 4 AND 5. CURVES SIMILAR TO THAT OF FIGURE 2 PLOTTED FROM OTHER SERIES OF PLATINGS

distilled water dilutions held in ice water. Growth would not ordinarily take place at this temperature and certainly not at such a rate as would be necessary to obtain the numbers found in



figure 1. The milk from which these organisms came did not itself show such an increase and in the milk the bacteria are in an environment much more suitable than in the dilution water. A dilution made from peptone, even at temperatures of 20°C. did not show an increase in two hours. Growth seems out of the question.

A study of the probable source of errors was made from another point of view. Instead of making a number of plates from one dilution, a single plate was poured from each of 50 dilution bottles. The agar was poured into the plate as soon as the dilution was added, the plates incubated at 37°C. and counted after forty-eight hours. In this way the time factor entering into the

TABLE 1

TRIAL	LOW	HIGH	MEAN	STANDARD DEVIATION	C.V.
1	1,500	3,700	2,436 $\pm$ 486	510 $\pm$ 34.3	20.9%
2	1,400	4,500	2,714 $\pm$ 649	680 $\pm$ 45.8	25.1
3	1,500	3,200	2,082 $\pm$ 429	450 $\pm$ 30.3	21.0
4	1,000	4,500	2,648 $\pm$ 611	640 $\pm$ 43.2	24.0
5	2,000	4,300	2,690 $\pm$ 448	470 $\pm$ 30.2	17.0
6	1,900	4,400	3,862 $\pm$ 334	350 $\pm$ 22.5	9.0
7	1,600	3,600	2,380 $\pm$ 363	380 $\pm$ 25.6	16.0
8	1,400	3,600	2,149 $\pm$ 410	430 $\pm$ 29.0	20.0
9	2,400	4,700	3,468 $\pm$ 448	470 $\pm$ 31.7	13.5

previous study was entirely lacking. There were no instances of the typical increase as noticed when the plots were made from the same dilution bottle. Table 1 shows the statistical data obtained, and it is interesting to note the low coefficients of variation obtained, although it must be remembered that the chance of error was increased by having each plate poured from a different dilution bottle. Since it might be of interest to graphically picture the results of this latter experiment, figure 6 is given to show the slight variation from the mean in each of those experiments. The abscissa is common to all trials, while the individual trials are indicated, each with its separate ordinate, which represents the count. From these data it was evident that the cause of the increase in colony count was associated with the time

of holding of the milk-water dilution. Something takes place in the dilution which causes an increase in the colonies on the plate.

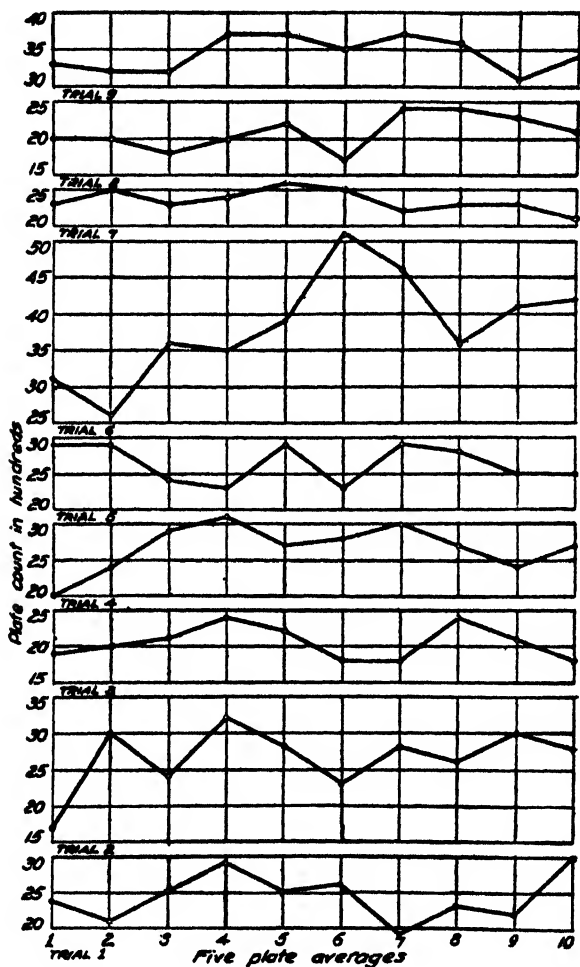


FIG. 6. FIVE PLATE AVERAGES FOR NINE SAMPLES OF MILK USING ONE DILUTION BOTTLE FOR EACH PLATE

That bacteria are present in milk as clumps or groups is a well known fact, recognized by all as a factor to be taken into consideration when the direct count is compared to the plate

count. This clumping does not refer to the strepto-form of cell grouping. Robertson (4) made a careful study of these clumps, finding that the plate count is really an estimate of the number of colonies that develop from such clumps, rather than from individuals. He found that groups of four to five occurred with the greatest frequency.

The reason that bacteria occur in this form is hard to explain. There are several possible explanations:

1. That the effect is due to the agglutination of the bacteria by the antibodies, which have been demonstrated time and again.

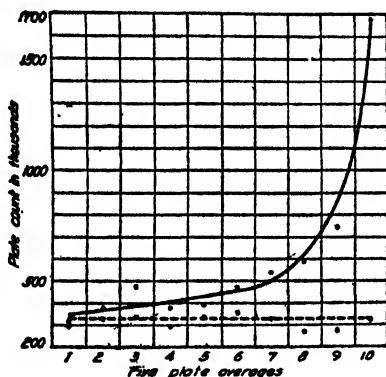


FIG. 7. FIVE PLATE AVERAGES OF COLONY COUNTS ON PLATES MADE FROM DISTILLED WATER (SOLID LINE) AND FROM PHOSPHATE BUFFER SOLUTION OF pH 6.6 (BROKEN LINE)

2. That it is due to certain charges on the organism.
3. That it is due to an acid agglutination of the bacteria.

Since fresh milk is of a fairly constant hydrogen ion concentration and since there is a more or less typical flora common to milk of this hydrogen ion concentration, this study was continued in an endeavor to discover what was the cause of the upward trend. The thought behind the newer work was that the dilution of the milk would change the pH of the milk, which in turn might lessen the forces holding the bacteria in the clumps.

With this in mind, two types of dilution water were prepared. One was distilled water, the other was a buffered solution, ad-

justed to pH 6.6 as suggested by Clark (5). Several samples of milk were used, dilutions being made as before, after a direct microscopic examination of the milk. Fifty plates were poured from each of the dilutions, the plates being numbered to deter-

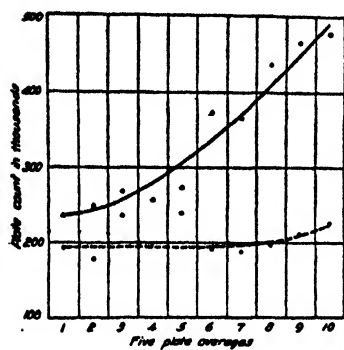


FIG. 8

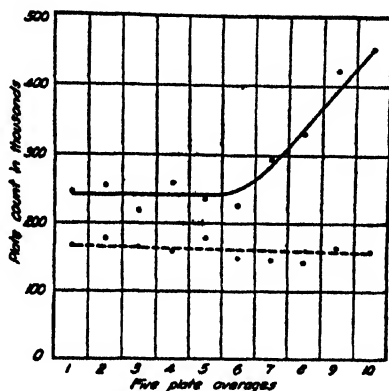


FIG. 9

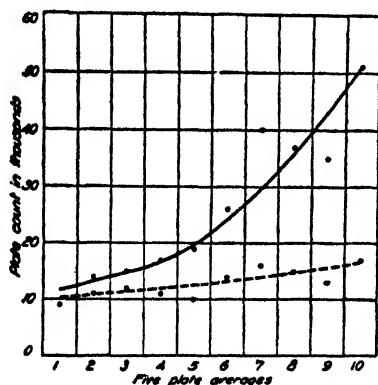


FIG. 10

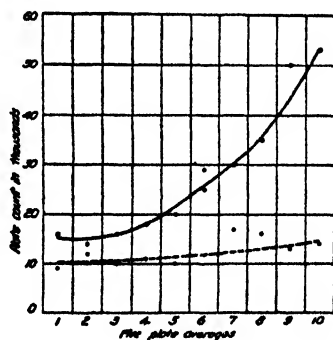


FIG. 11

FIGS. 8, 9, 10 AND 11. CURVES SIMILAR TO THAT OF FIGURE 7 PLOTTED FROM OTHER SERIES OF PLATINGS

mine the effect of holding time. The data are of considerable interest, and are shown in the figures 7 to 11. Here, as above, the running averages of 5 plates were plotted for the plates made from the two types of dilutions. The solid line represents the

counts derived from plates made from the distilled water, the dotted line represents the counts obtained from the plates made from the phosphate buffers of pH 6.6. This has been demonstrated many times during this study. At no time has the count in the buffer solution shown an appreciable rise, and at no time has the unbuffered solution failed to show the effect. As mentioned above, this upward trend is found in distilled water that has been kept in ice water, which would tend to disprove the contention that this is a growth effect. It further disproves the growth theory and at the same time substantiates the buffered solution theory. A 1 per cent peptone solution adjusted to pH 6.6 has been used with substantially the same results.

A statistical analysis of the data from which figure 7 was derived discloses that the plates made from the unbuffered dilution had a mean of  $592,000 \pm 269,000$  and a coefficient of variation of 67 per cent, while those from the buffered solution had a mean of  $310,000 \pm 31,000$  with a coefficient of variation of but 17 per cent.

It is quite evident that the milk-water dilution is a considerable factor in the plating of milk, and that more attention should be given it. It would be well to call attention at this point to the directions found in Standard Methods that "the work should be so planned that no more than fifteen minutes shall elapse after the dilution of the milk and before the agar is poured."

Too frequently does time elapse between dilution of the milk and the addition of the agar to the plates. If this time factor is more than fifteen minutes, it is easy to explain possible discrepancies in counts.

A study of figure 1 will explain this better. Let plate 60 be chosen for one of a pair of plates, with its colony count of 80,000. It would be well to recall that about an hour has elapsed from the time the dilution was made. If plate 59 is to be considered the duplicate of plate 60, its colony count is 600,000, while that of plate 61 is 850,000. The explanation for this in the light of the present study, is that plates 59 and 61 were made from less large clumps, while by chance plate 60 was seeded with clumps about the size originally found in the dilution bottle. (See plates 1-40.)

Attempts to actually see the dispersion of the clumps by means of staining were unsuccessful. The evidence is entirely circumstantial, that the dispersion effect is the correct solution of the phenomenon, but the evidence seems to be substantial.

An attempt was made to use bile to disperse the clumps to an even greater extent. This was unsuccessful.

#### CONCLUSION

It has been shown that:

The statistical studies upon the plate method are subject to invalidating effects.

A significant rise in colony formation takes place upon allowing the milk-water dilution to stand.

The addition of a phosphate or a peptone buffer of pH 6.6 prevents this.

This effect is due to dispersion of clumps.

The dilution bottle is of a hitherto unsuspected importance in the process of plating.

Standard Methods should be rigidly adhered to in respect to the time interval between making of the dilution and the addition of the agar to the plate.

#### REFERENCES

- (1) BREED, R. S. 1926 Standard methods of milk analysis, bacteriological methods. Amer. Jour. Pub. Health, xvi, 811.
- (2) WRIGHT, W. H., AND THORNTON, H. R. 1927 How accurate is the quantitative plate count? Jour. Bacteriol., xiii, 63.
- (3) MUDGE, C. S. AND LAWLER, B. M. 1928 Is the statistical method applicable to the bacterial plate count. Jour. Bacteriol., xv, 207.
- (4) ROBERTSON, A. H. 1921 The relation between bacterial count from milk as obtained by microscopic and plate methods. N. Y. Agric. Exp. Sta. Tech. Bul. 86.
- (5) CLARK, W. M. 1922 The Determination of Hydrogen Ions. Williams & Wilkins Co., Baltimore, p. 114.

# DISTRIBUTION AND GROWTH OF BACTERIA IN BUTTER\*

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In 1927 it was shown by one of the authors that the number of moisture droplets in butter is very large, ranging from about 10 to 18 billions per gram of butter. In butter from ripened cream we seldom find more than 50 millions of microorganisms, in pasteurized sweet-cream butter sometimes only 10,000 are present in 1 gram of butter. Thus it is evident that not all moisture droplets in butter contain bacteria; the majority must be free from them; sterile drops are formed in the churning process. How small a percentage of the total moisture is infected may be seen from the following calculations and from one of our first experiments.

If we know the number and the size of the moisture droplets of the butter and the number of bacteria per cubic centimeter in the buttermilk, we can easily compute the percentage of the infected moisture. The distribution of the moisture in butter can be measured accurately enough. The method of Boysen (1) is as follows:

All moisture droplets are measured and counted directly under the microscope in a thin film of butter. They are divided into 12 groups, the first comprising all droplets up to  $3\mu$  in diameter, the next from 3 to  $5\mu$ , then 5 to  $10\mu$ , 10 to  $15\mu$ , 15 to  $25\mu$ , 25 to  $35\mu$  diameter and so on up to  $100\mu$ . Droplets larger than these cannot be seen and accurately counted under the microscope as a layer of butter thicker than  $100\mu$  or 0.1 mm. is not sufficiently transparent.

The groups from 0 to  $15\mu$  in diameter are counted in a thin layer of butter in special counting chambers (Zeiss) of  $10\mu$  depth at a magnification of 900 diameters (Zeiss eyepiece  $15\times$  and objec-

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tive  $60\times$ , dry system). A net-micrometer in the eyepiece (fig. 1) allows one to measure each droplet and to count all droplets of a certain size in a given field. In the above-mentioned combination of lenses, the length of one part of the scale is exactly one micron, the largest square then holds  $100 \times 100 = 10,000$  square microns, and as the depth of the film is 10 microns, the measured space is 100,000 cubic microns. Two fields in each of 10 preparations are examined in this way and the numbers are calculated for 1 gram of butter.

The larger drops are measured and counted at a magnification of 150 diameters (same eyepiece and objective  $10\times$ ) in a counting-chamber of  $100\mu$  depth. Four of the largest squares are

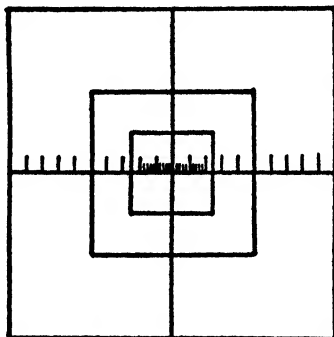


FIG. 1. NET-MICROMETER FOR EYEPIECE

examined in 10 preparations each, the total examined space thus being exactly 1 gram of butter.

The average volume of the droplets of each group has been determined, and by multiplying the number of droplets of each group with the corresponding average volume, the whole water volume is obtained. This volume means in the case of salted butter, practically also its weight. However, in unsalted butter, about 5 per cent of the computed water volume has to be added to change cubic centimeters into grams on account of the different densities.

The percentage of moisture in butter found in this way is usually smaller than the percentage found by chemical analysis.



This difference probably represents the drops larger than  $100\mu$  in diameter. There is some evidence for this in the fact that in some experiments in which butter was highly overworked this difference decreased to an amount which lies within the limits of error. Practically all moisture could be accounted for by this method; the percentage in the smaller droplets increasing continuously as the working process went on.

TABLE 1  
*Calculation of the amount of infected moisture in butter A*

DIAMETER OF MOISTURE DROPLETS	AVERAGE VOLUME OF ONE DROPLET	NUMBER OF DROPLETS IN 1 GRAM OF BUTTER	VOLUME OF ALL DROPS IN 100 GRAMS OF BUTTER	PERCENTAGE OF INFECTED DROPS	GRAMS OF INFECTED MOISTURE IN 100 GRAMS OF BUTTER
$\mu$	cubic microns		cc.		
0-3	3.59	9,852,000,000	3.675	0.000,018	Under 1 mgm.
3-5	33.5	253,000,000	0.893	0.000,168	Under 1 mgm.
5-10	221	39,000,000	0.905	0.001,100	Under 1 mgm.
10-15	1,020	7,000,000	0.751	0.005,010	Under 1 mgm.
15-25	4,190	360,000	0.159	0.021	Under 1 mgm.
25-35	14,100	90,000	0.133	0.070	Under 1 mgm.
35-45	33,500	32,000	0.112	0.168	Under 1 mgm.
45-55	65,500	24,000	0.165	0.328	0.001 gram
55-65	113,000	18,000	0.213	0.565	0.001 gram
65-75	180,000	6,000	0.113	0.900	0.001 gram
75-85	268,000	2,000	0.056	1.340	0.001 gram
85-95	382,000	2,000	0.080	1.910	0.002 gram
100 and more*			5.365		3.317 grams
Total.....		10,000,000,000	12.620		3.323 grams

\* = extrapolated.

As the percentage for each of the larger groups becomes almost constant, it is possible to extrapolate the distribution of the drops larger than  $100\mu$ , and the results are rather plausible. In the most unfavorable cases the error of this method may be as high as 0.46 per cent of the butter weight. Table 1 gives the distribution of moisture in one of the samples of the first experiment, and table 2 shows the average distribution of moisture in German butter, and the method of extrapolation.

TABLE 2

*Influence of the bacterial content of cream upon the amount of moisture remaining sterile in butter*

DIAMETER OF DROPLETS	AVERAGE NUMBER OF MOISTURE DROPLETS PER GRAM OF BUTTER	PER CENT OF MOISTURE IN BUTTER	GRAMS OF MOISTURE INFECTED IN 100 GRAMS OF BUTTER IF THE BACTERIA COUNT OF BUTTERMILK AMOUNTS TO						
			1 billion	100 million	10 million	1 million	100 thousand	10 thousand	1 thousand
0-3	11,068,000,000	3.97	0.014	0.001	0.000	0.000	0.000	0.000	0.000
3-5	180,700,000	0.61	0.020	0.002	0.000	0.000	0.000	0.000	0.000
5-10	60,230,000	1.33	0.293	0.029	0.003	0.000	0.000	0.000	0.000
10-15	11,570,000	1.18	1.180	0.120	0.012	0.001	0.000	0.000	0.000
15-25	600,000	0.25	0.25	0.105	0.010	0.001	0.000	0.000	0.000
25-35	160,000	0.23	0.23	0.23	0.032	0.003	0.000	0.000	0.000
35-45	69,000	0.23	0.23	0.23	0.077	0.008	0.001	0.000	0.000
45-55	29,000	0.19	0.19	0.19	0.124	0.012	0.001	0.000	0.000
55-65	16,000	0.18	0.18	0.18	0.18	0.020	0.002	0.000	0.000
65-75	12,000	0.22	0.22	0.22	0.22	0.039	0.004	0.000	0.000
75-85	6,500	0.17	0.17	0.17	0.17	0.046	0.005	0.000	0.000
85-95	5,100	0.19	0.19	0.19	0.19	0.073	0.007	0.001	0.000
100	3,800	0.20	0.20	0.20	0.20	0.105	0.011	0.001	0.000
110	2,900	0.20	0.20	0.20	0.20	0.139	0.014	0.001	0.000
120	2,200	0.20	0.20	0.20	0.20	0.181	0.018	0.002	0.000
130	1,700	0.20	0.20	0.20	0.20	0.20	0.023	0.002	0.000
140	1,400	0.20	0.20	0.20	0.20	0.20	0.030	0.003	0.000
150	1,140	0.20	0.20	0.20	0.20	0.20	0.035	0.004	0.000
160	940	0.20	0.20	0.20	0.20	0.20	0.043	0.004	0.000
170	775	0.20	0.20	0.20	0.20	0.20	0.051	0.005	0.001
180	655	0.20	0.20	0.20	0.20	0.20	0.061	0.006	0.001
190	560	0.20	0.20	0.20	0.20	0.20	0.072	0.007	0.001
200	480	0.20	0.20	0.20	0.20	0.20	0.084	0.008	0.001
210	415	0.20	0.20	0.20	0.20	0.20	0.097	0.010	0.001
220	360	0.20	0.20	0.20	0.20	0.20	0.111	0.011	0.001
230	315	0.20	0.20	0.20	0.20	0.20	0.127	0.013	0.001
240	277	0.20	0.20	0.20	0.20	0.20	0.145	0.015	0.001
250	245	0.20	0.20	0.20	0.20	0.20	0.163	0.016	0.002
260	218	0.20	0.20	0.20	0.20	0.20	0.184	0.018	0.002
270	195	0.20	0.20	0.20	0.20	0.20	0.200	0.021	0.002
Moisture infected .....			6.777	5.277	4.628	3.628	1.499	0.147	0.011
Moisture sterile .....			5.583	7.083	7.732	8.732	10.871	12.213	12.349
Total moisture .....		12.36	12.36	12.36	12.36	12.36	12.36	12.36	12.36
Per cent of total moisture sterile .....			45.2	57.4	62.5	71.0	88.0	99.0	(100)

The computation of the amount of infected moisture in butter is shown in table 1. Butter A was neither washed nor salted and its plasma, i.e. the fat-free part of the butter, must be of the same approximate composition as the corresponding buttermilk and, of course, must have the same approximate bacterial count which was in this case 50,000 per cubic centimeter, the cream being pasteurized and unripened. One gram of the butter contained 9,852,000,000 droplets of the smallest group, under  $3\mu$  in diameter which amounted in total to 0.0368 gram of moisture. As this moisture contained 50,000 bacteria per cubic centimeter, there are in these smallest droplets  $0.0368 \times 50,000 = 1,840$  bacteria. This simply means that not more than 1,840 out of 9.8 billion droplets can be infected. The rest, 99.99,998 per cent, must be sterile.

As the size of the droplets increases, the amount of the moisture infected also increases. This is shown in table 1 where the calculation has been carried out for all 12 groups, including the extrapolation. The general formula for the percentage of infected moisture is  $\frac{W \cdot N}{D}$ , in which  $W$  is the amount of water of each group in 100 grams of butter,  $N$  is the bacterial count of 1 cc. moisture in the butter and  $D$  is the number of drops of the group in question. Then the amount of the infected moisture in 100 grams of butter is  $\frac{W \cdot W \cdot N}{100 \cdot D}$ , or as  $\frac{W}{100 \cdot D}$  equals the average volume of that group:  $W \cdot N \cdot \text{volume}$ .

In our sample of butter, 3.32 grams of moisture in 100 grams of butter are infected, i.e., 26.4 per cent of the total moisture content. In table 2, this calculation has been carried out for various bacterial counts in the buttermilk on the basis of an average moisture distribution as found in German butter. It shows that in butter from very sour cream, about 50 per cent of the total moisture is sterile, while in well pasteurized sweet-cream butter almost 99 per cent is free from bacteria. All practical cases probably lie between these two limits.

It does not seem very reasonable and probable that in churning a highly infected cream, so large a part of the new product be-

comes sterile. Yet it is a fact, as these calculations are based upon well established data only. And it does not seem so surprising if we consider that even in a full grown culture of *Streptococcus lactis* with a billion bacteria per cubic centimeter every cell has still a living space of 1000 cubic microns, while the smallest moisture droplets in butter average only 3.59 cubic microns.

This is also evident by the low count of bacteria in butter which has remained unexplained so far; the largest number of bacteria per gram of butter has been recorded by Orla-Jensen (5) to be 59,000,000, by Teichert (10) 22,000,000, by Lorenz (4) 49,000,000, by Schmidt (9) 27,000,000, by Sayer, Rahn and Farrand (8) 26,000,000. The lactic acid organisms may multiply in milk to 1,000,000,000 cells per cubic centimeter, as may also the colon organisms, *Pseudomonas fluorescens* and other putrifiers. So it would be expected that in butter with 15 per cent of moisture, we should find about 15 per cent of 1,000,000,000, i.e., 150,000,000 bacteria per gram. The fact that never more than 59,000,000 have been observed, is an indication that conditions of growth in butter must be different from those in milk. The computation of the bacteria-free moisture droplets gives a simple explanation for this difference.

The question arises as to how this uneven distribution of bacteria in the moisture influences the keeping quality of the butter. According to the two conceptions of the structure of butter there are two possibilities [Rahn (6)]. The theory of the inversion of phases claims that all water droplets are entirely separated from each other, the fat being the continuous phase. In this case decomposition of butter could take place only to the extent in which its moisture is infected. It is not entirely impossible however that some slight exchange might take place through the fat, as, e.g., lactic acid formed in the larger infected drops may diffuse to the sterile ones because lactic acid is slightly fat-soluble, thus giving the bacteria a new chance to ferment more lactose. According to the foam theory of the churning process all drops are connected by a very thin network consisting of the membranes of fat globules which are thick enough to serve as diffusion channels but which are too narrow to let bac-

teria pass, the smallest of which would be at least 10 to 20 times as large as these connecting layers. In this case a slow diffusion of the products would be possible, thus causing butter to be decomposed to a higher degree than would be expected according to the percentage of infection. The two structural possibilities are illustrated in the textbook of Rahn and Sharp (7, p. 111).

To decide between the two possibilities, comparisons have been made between butter and true solidified emulsions of skimmilk in butterfat. The other questions dealt with here are those of the influence of washing, working and salting the butter. Our preliminary experiments are omitted as our efforts to separate the small from the large drops in butter failed.

In all series of experiments the formation of acid was used as a measure of bacterial decomposition and it was determined by titrating with  $N/14$  NaOH with phenolphthalein as indicator. In the first two experiments the hydrogen-ion concentration was determined also but was later omitted as the exact amount of lactic acid cannot be determined in this way in such highly buffered solutions. In all butter samples the distribution of the moisture droplets was determined according to the method described above. Furthermore, the bacterial content was determined both in the butter and in the buttermilk. For the determination of the acid, about 100 grams of butter were melted at  $70^{\circ}$  to  $80^{\circ}\text{C}$ . until the liquid fat had separated entirely from the plasma, the plasma was then mixed before titration.

In experiment I a quantity of pasteurized cream was divided into four parts and churned separately in the same churn. One part was neither washed nor salted, another washed but not salted. The two other parts were salted, one of them being washed. Of each of these four portions one-half was normally worked whereas the rest was overworked, thus giving eight different samples of butter which were stored, together with a sample of the buttermilk, under the same conditions at room temperature.

Table 3 shows the distribution of the water droplets in these eight samples and the amount of infected moisture as calculated in the described way. Unfortunately, a finer distribution of the

water by overworking was obtained only in the first case. In the other samples the number of small droplets was increased but so were the larger drops because more water had been worked into the butter during the overworking process. Thus the percentage of infected moisture appears a little too high because the increase in moisture after overworking did not come from the buttermilk but from the washwater which did not contain as many bacteria.

TABLE 3  
*Distribution of moisture in the samples of experiment I*

TREATMENT OF THE BUTTER			SAMPLE	AMOUNT OF MOISTURE IN DROPLETS OF:			TOTAL MOISTURE	INFECTED MOISTURE
				0-15 $\mu$	15-100 $\mu$	Over 100 $\mu$ diameter		
				per cent	per cent	per cent	per cent	per cent
Unsalted.....	Unwashed {	Normally worked	A	6.22	1.03	5.37	12.62	26.4
		Overworked	B	8.13	0.93	4.04	13.10	17.0
	Washed {	Normally worked	C	5.95	1.12	4.95	12.02	23.0
		Overworked	D	6.84	0.81	5.57	13.22	30.0
Salted.....	Unwashed {	Normally worked	E	5.47	2.09	3.71	11.27	4.8
		Overworked	F	6.10	0.86	5.69	12.65	32.3
	Washed {	Normally worked	G	5.88	1.52	3.50	10.90	7.0
		Overworked	H	5.77	1.32	5.31	12.40	22.0

In the case of samples A and B, unsalted and unwashed butter, the composition of the control plasma is exactly like that of the buttermilk. It is therefore possible to compare decomposition directly. The formation of acid in the plasma of the butter samples and in the buttermilk should be the same if the changed physical conditions of the moisture in the butter had no influence. Table 4 shows that the buttermilk sours much faster than does the equal amount of moisture present in the butter. For exact comparison we have to consider the *increase* in acidity instead of the total acidity. In three days the buttermilk increased its

acidity 0.7266 per cent, while the corresponding increases in the butter samples A and B were only 0.3086 and 0.1736 per cent (calculated as lactic acid). This is about 42 and 24 per cent of the increase in the buttermilk, which must be considered as

TABLE 4  
*Acid formation in the plasma of the butter samples of experiment I*

	NOT SALTED				SALTED				BUT- TER- MILK
	Unwashed		Washed		Unwashed		Washed		
	Normal- ly worked	Over- worked	Normal- ly worked	Over- worked	Normal- ly worked	Over- worked	Normal- ly worked	Over- worked	
	A	B	C	D	E	F	G	H	
	Infected moisture in percentage of total moisture in samples								
	26.4	17.0	23.0	30.0	4.8	32.3	7.0	22.0	100
Acidity in percentage of lactic acid									
After 0 day .....	0.2508	0.2508	0.1222	0.1029	0.1993	0.1993	0.1190	0.1093	0.2572
After 1 day .....	0.2926	0.2765	0.1610	0.1180	0.2251	0.2251	0.1670	0.1225	0.8745
After 2 days .....	0.3729	0.3022							0.9774
After 3 days .....	0.5594	0.4244	0.1870	0.1542	0.2000	0.2775	0.1290	0.1227	0.9838
After 11 days .....			0.2260	0.1870	0.2630	0.2380	0.1740	0.1290	
Increase in acidity; percentage of lactic acid									
After 1 day .....	0.0418	0.0257	0.0388	0.0151	0.0258	0.0258	0.0480	0.0132	0.6173
After 2 days .....	0.1221	0.0514							0.7202
After 3 days .....	0.3086	0.1736	0.0648	0.0513	0.0007	0.0782	0.0100	0.0134	0.7266
After 11 days .....			0.1038	0.0841	0.0637	0.0380	0.0550	0.0197	
pH									
After 0 day .....	5.97	5.95	6.39	6.41	5.45	5.50	5.75	5.80	5.90
After 1 day .....	5.72	5.70	6.29	6.40	5.51	5.50	5.74	5.75	4.50
After 2 days .....	5.48	5.65	6.18	6.36	5.33	5.55	5.38	5.80	4.15
After 3 days .....	5.52								4.20
After 11 days .....			5.51	5.56	5.51	5.63	5.55	5.78	

the highest possible increase. The acid formation is less in the butter than in the buttermilk, yet it is higher than would be expected. The percentage of infected moisture is 26.4 per cent in the case of butter A and 17 per cent in the case of butter B

which would account only for about two-thirds of the actually observed increase. Therefore fermentation does not seem to be limited to the infected drops only.

The other samples show the effect of washing and salting; this is dealt with later. They cannot be compared directly with A and B and the buttermilk because the composition of their plasma is not the same as that of the free buttermilk.

In order to get complete comparisons, the experiment was repeated; this time the pasteurized cream was inoculated with a very small amount of starter, and instead of four samples only

TABLE 5  
*Distribution of the moisture in the samples of experiment II*

TREATMENT OF THE BUTTER			SAMPLE	PERCENTAGE OF MOISTURE IN DROPS WITH A DIAMETER OF:			TOTAL MOISTURE	INFECTED MOISTURE
				0-15 $\mu$	15-100 $\mu$	Over 100 $\mu$		
Unsalted.....	Unwashed	Normally worked	I	7.72	1.11	1.75	10.58	7.7
	Washed	Normally worked	II	7.65	0.61	4.09	12.35	29.2
		Overworked	III	7.92	0.49	6.55	14.96	41.0
Salted.....	Washed	Normally worked	IV	5.01	1.38	5.38	11.77	36.8
		Overworked	V	7.12	0.67	5.48	13.27	31.5

one was left unwashed. In order to obtain a "buttermilk" comparable with the plasma in the washed and salted, and also in the washed and unsalted butter, several pounds of each of these samples were melted at a temperature not over 40°C. and the plasma separated. This liquid was approximately comparable with the moisture present in the respective butter samples in chemical as well as in bacteriological respects. All samples were printed and immediately brought into a room at 22°C. where they were kept together with the buttermilk samples. The buttermilk contained about 200,000 bacteria per cubic centimeter. The distribution of the moisture droplets is given in table 5 and the acid formation in table 6.



Unfortunately, also in this case, overworking did not decrease the moisture content and did not make the distribution finer, thus giving again a wrong number for the percentage of infected

TABLE 6  
*Acid formation in the plasma of the butter samples of experiment II*

	NOT SALTED			SALTED		BUT- TER- MILK	PLASMA OBTAINED FROM SAMPLES	
	Unwashed	Washed		Washed				
	Normally worked	Nor- mally worked	Over- worked	Nor- mally worked	Over- worked			
	I	II	III	IV	V		II + III	IV + V
	Infected moisture in percentage of total moisture in samples							
	7.7	29.2	41.0	36.8	31.5	100	100	100
Acidity in percentage of lactic acid								
After 0 day.....	0.4050	0.2060	0.1675	0.2180	0.1994	0.4960	0.3540	0.3540
After 2 days.....	0.3870	0.2830	0.2325	0.2380	0.2265	0.7735	0.5670	0.3355
After 4 days.....	0.4960	0.3280	0.2710	0.2900	0.2610	0.8770	0.6570	0.3415
After 7 days.....	0.5210	0.3600	0.2970	0.3180	0.2610	0.9250	0.8380	0.3280
After 32 days.....	0.6620	0.4735	0.4370	0.3000	0.2950			
After 64 days.....	0.6623	0.5401	0.4951	0.3215	0.2765			
Increase in acidity; percentage of lactic acid								
After 2 days.....	(-0.0180)	0.0770	0.0650	0.0200	0.0271	0.2775	0.2130	(-0.0185)
After 4 days.....	0.0910	0.1220	0.1035	0.0720	0.0616	0.3810	0.3030	(-0.0125)
After 7 days.....	0.1160	0.1540	0.1295	0.1000	0.0616	0.4290	0.4840	(-0.0260)
After 32 days.....	0.2570	0.2675	0.2695	0.0820	0.0956			
After 64 days.....	0.2573	0.3341	0.3276	0.1035	0.0771			
pH								
After 0 day.....	4.95	5.30	5.40	4.95	5.02	5.02	5.11	5.00
After 2 days.....	5.06	4.89	5.11	4.63	3.67	4.11	4.21	4.94
After 4 days.....	4.92	5.14	5.11	4.94	5.01	4.08	4.07	5.05
After 7 days.....	4.86	5.18	4.96	5.03	5.03	4.20	3.84	3.44
After 32 days.....	4.58	4.72	4.50	5.48	5.95			
After 64 days.....								

moisture. These results will be discussed later. The three unsalted samples show a distinctly slower decomposition than the buttermilk or the separated plasma. But in all cases

finally the amount of acid found in butter is 62 to 69 per cent of that of the corresponding free moisture, while the percentage

TABLE 7

*Distribution of moisture in the butter samples of experiment III*

SAMPLES	PERCENTAGE OF MOISTURE IN DROPLETS WITH A DIAMETER OF			TOTAL MOISTURE	INFECTED MOISTURE
	0-15 $\mu$	15-100 $\mu$	Over 100 $\mu$		
				<i>per cent</i>	<i>per cent</i>
Normally worked .....	8.29	0.91	4.77	13.97	37.6
Thoroughly worked .....	9.08	0.68	4.21	13.97	33.4
"Creamed" .....	9.95	0.52	3.50	13.97	27.2

TABLE 8

*Acid formation in the plasma of the butter samples of experiment III*

	NORMALLY WORKED	THOR- OUGHLY WORKED	"CREAMED"	PLASMA OB- TAINED BY MELTING
	Sample N	Sample O	Sample C	
Acidity in percentage of lactic acid				
After 0 day .....	0.055	0.055	0.055	0.055
After 1 day .....				0.296
After 2 days .....	0.129	0.071	0.064	0.456
After 5 days .....				0.520
After 6 days .....	0.238	0.148	0.071	0.540
After 8 days .....				0.559
After 12 days .....	0.289	0.161	0.084	1.138
After 24 days .....	0.335	0.196	0.116	1.010
After 42 days .....	0.412	0.232	0.116	
After 60 days .....	0.472	0.386	0.180	1.090
Increase in acidity; percentage of lactic acid				
After 2 days .....	0.074	0.016	0.009	0.401
After 6 days .....	0.183	0.093	0.016	0.455
After 12 days .....	0.234	0.106	0.029	1.083
After 24 days .....	0.280	0.141	0.061	0.955
After 42 days .....	0.357	0.177	0.061	
After 60 days .....	0.417	0.331	0.125	1.035

of infected moisture amounts only to 8 to 41 per cent. Here we have the same result as in the first experiments; more droplets

are decomposed than are infected. Both of the salted samples, strange to say, show a slight increase in titrable acidity and a decrease in hydrogen-ion concentration. The comparable moisture also shows a decrease in titrable acidity. However interesting this behavior may be, it does not help to solve the problem of the bacterial distribution and therefore it may be left out of consideration.

Another experiment was made in order to get an overworked butter without an increased moisture content. Pasteurized sweet cream was inoculated with lactic acid bacteria and churned at once. Of the resulting butter one part was worked normally while another part was overworked and an equal portion brought to a creamy consistency in a mortar with a pestle. All samples were treated in the same way and again a certain amount of free plasma was obtained by melting. All three samples being alike, save the mechanical treatment, one plasma sample was sufficient for all three. The results are shown in tables 7 and 8.

#### THE MEANING OF THE STERILE MOISTURE

In all experiments in which a noticeable decomposition of the plasma took place, the acid formation went further than would be expected from the amount of infected moisture. The seven samples of unsalted butter of the first two experiments give the most information on this point. A comparable moisture existed for all except samples C and D. Here the undiluted buttermilk was taken for the calculations, though the butter was washed. But acid formation is slowed down only very little by diluting even with equal amounts of water. (See the plasma in table 6.)

In table 9 the increase in acidity in 100 cc. of moisture *in* the butter is calculated in percentages of the same moisture *outside* of the butter. In each case, 100 is the number which would be obtained if the liquid was not distributed in the form of small droplets but was present as the continuous phase, as buttermilk or diluted buttermilk.

If there were no possibility of exchange between infected and sterile droplets, only as much acid could be formed in the butter

as corresponds to the percentage of infected plasma. In the case of butter A, the amount of acid in the butter should, at any time, be 26.4 per cent of the amount of acid in the buttermilk. The table shows that this is not the case. In the first days, however, this holds fairly true. In the unwashed samples this limit is exceeded already on the third day, while in the washed samples this does not take place until after the twelfth day.

In the washed samples, however, we have to consider that the value for the infected moisture is too high. During the washing

TABLE 9  
*Acidity of the plasma in percentage of total acidity possible*

SAMPLE	INFECTED PLASMA IN PERCENTAGES OF TOTAL	ACIDITY OF PLASMA IN PERCENTAGES OF TOTAL ACIDITY POSSIBLE, ON THE FOLLOWING DAYS													
		1	2	3	4	6	7	11	12	24	32	42	60	64	
Unwashed butter															
A	26.4	7	17	42											
B	17.0	4	7	24											
I	7.7		0		24		27				62			62	
Washed butter															
C	23.0	6		10				14							
D	30.0	2		6				12							
II	29.2		36		41		32				55			69	
III	41.0		31		34		27				56			68	
N	37.6		18			38			23	27		35	41		
O	33.4		4			19			10	14		17	32		
C	27.2		2			3			3	6		6	12		

process, buttermilk with its high bacterial count is replaced by water with a low count. Yet this error is not so great that it can explain the slower acid formation.

Sooner or later, in every butter, more acid is formed than corresponds to the infected plasma. Some kind of exchange must then take place between the infected and the sterile droplets. There are again the two possibilities. If all droplets are connected with each other as Rahn (6) believes this would mean a slow diffusion. Rahn found that salt from a 25 per cent brine

diffused 15 mm. into unsalted butter in three months. i.e.,  $15,000\mu$  in ninety days, or  $167\mu$  in one day, or  $7\mu$  per hour. The average distance between the water droplets amounts to about  $5\mu^1$  so that a rather complete exchange seems possible, though, of course, a 25 per cent brine diffuses much faster than a 0.1 per cent lactic acid.

However, this conception of Rahn of the structure of butter is not generally accepted. Hunziker (3) assumes that all water droplets lie separately in butter. In this case, an exchange would be possible only if the lactic acid could diffuse through the continuous fat walls. This is not quite impossible as lactic acid is soluble in fat. A simple experiment was tried to decide this question. Solutions of 5 and 1 per cent lactic acids were kept separated from pure water by a 5 mm. film of butterfat. After more than two months no trace of acid could be found in the outside water.

At the same time this problem was worked on from another point of view. Artificial "butter" was prepared by emulsifying skimmilk, inoculated with lactic acid bacteria, in melted, filtered butterfat. It is rather certain that in such an emulsion all water droplets are single without any connections between each other. The emulsions, churned in a glass churn, were solidified rapidly with cold water and were then treated just like the butter samples of the previous experiments.

In the first experiment the number of lactic acid bacteria was too low, thus causing only 0.66 per cent of the plasma to be infected. This emulsion did not show any change at all until finally it molded. In a second experiment one emulsion was made to contain more bacteria than the other. This time, however, the emulsions were rather coarse and thus the calculations of the infected plasma are more inaccurate than usual. Yet the data in table 10 show that after ten days, and in one case after sixteen days, no further increase in decomposition takes place for some

<sup>1</sup> After the formula:  $X = d \left( \sqrt[3]{\frac{74.04}{W}} - 1 \right)$  in which  $d$  is the average diameter of the water droplets and  $W$  the water content in 100 grams of butter (Rahn and Sharp (7, p. 178)).

time. The irregularities in the lower part of the table were found to be due to the development of high acid producing organisms one of which formed, in milk, 1.9 per cent of lactic acid in four weeks. It seems, therefore, correct to neglect the figures for the sixty-fourth day because the three preceding data are as constant as can be expected with this method of determination. The con-

TABLE 10  
*Solidified emulsions of skimmilk in butterfat*  
A. Distribution of moisture

SAMPLE	NUMBER OF BACTERIA IN 1 CC. OF THE PLASMA	PERCENTAGE OF MOISTURE IN DROPLETS OF			TOTAL PERCENTAGE OF MOISTURE	PERCENTAGE OF INFECTED MOISTURE
		0-15 $\mu$	15-100 $\mu$	over 100 $\mu$		
E I	13,500	2.32	3.33	8.85	14.50	4.4
E II	740,000	2.12	2.39	9.49	14.50	63.4

B. Acid formation in percentage of lactic acid

	TOTAL ACIDITY				INCREASE IN ACIDITY			
	E I		E II		E I		E II	
	Emul-sion	Plasma	Emul-sion	Plasma	Emul-sion	Plasma	Emul-sion	Plasma
After 0 days.....	0.116	0.142	0.119	0.155				
After 2 days.....	0.142	0.650	0.213	0.760	0.026	0.508	0.094	0.605
After 4 days.....	0.161	0.785	0.200	0.875	0.045	0.643	0.080	0.720
After 10 days.....	0.318	0.900	0.357	0.978	0.202	0.758	0.238	0.823
After 16 days.....	0.446	0.926	0.569	0.945	0.330	0.784	0.450	0.790
After 32 days.....	0.320	0.910	0.588	1.350	0.204	0.768	0.449	1.195
After 64 days.....	1.440	1.800	1.100	2.070	1.324	1.658	0.981	1.915

stancy as such is perhaps the best proof for the absence of any diffusion processes in the emulsions.

The decomposition in the emulsions reaches 26 and 56 per cent of that of the corresponding free plasma, these figures lie in one case way above, in the other a little below the computed values. The calculations for the percentage of infected moisture are not so accurate in the first emulsion because of the low bacterial count (13,000 per cubic centimeter) and the very uneven and coarse distribution of the moisture droplets. Emulsion II

seems to indicate that in true emulsions the decomposition does not exceed the amount of infected moisture.

#### THE INFLUENCE OF WASHING THE BUTTER

Not until lately has it been possible to sufficiently explain the good effect of the washing of butter on its keeping quality. The chemical analyses of Hittcher (2) show that hardly half of the lactose and less than one fourth of the curd can be washed out. Yet in milk, diluted to two volumes, all decomposition processes occur almost as fast as they do in undiluted milk. Dilution as such does not give an explanation for the better keeping of washed butter.

Rahn and Sharp (7, p. 117) give the following physical explanation for it: The smallest moisture droplets in butter originate during the churning process, they simply fill the intermediate spaces between the fat globules and they therefore contain pure buttermilk. These droplets are entirely enclosed in the butter-granules and do not come in contact with the washwater at all. From the outside of the butter-granules, however, the washwater removes all milky parts very thoroughly, since butter is usually washed until the drain water is clear. The water incorporated during the washing and working processes forms the larger drops. In butter two entirely different kinds of moisture droplets are present, namely, small droplets of buttermilk which are mostly sterile because of their minute size, and large drops which consist of almost pure water and which contain a mixture of bacteria from the cream and from the water. Since all calculations have shown that most of the bacteria are present in the large drops (see tables 1 and 2) the result of washing is that the food is present in the small droplets and the bacteria in the large ones, thus the bacteria being separated from their food. For this reason, protection of the butter by washing is so efficient, provided, of course, that the washwater is good. If the water contains lipolytic bacteria, e.g., *Ps. fluorescens*, the danger is greater, for in the large drops not enough lactose is present to allow of a strong acid formation which might retard lipolysis.

The assumption of Rahn and Sharp seems to be proved by these experiments. In table 11 are shown the increases in acidity in three samples of unwashed butter as compared with the washed control samples. All of these samples were unsalted.

The first two pairs show how acid formation is retarded in the washed samples. Butter II and III do not show this decrease, probably because of an incorrect determination of the initial acidity in sample I; for this butter shows a decrease in acidity after two days which seems quite improbable. The increases in acidity after the second day are:

In sample I.....	0.1090	0.1340	0.2750	0.2753
In sample II.....	0.0450	0.0770	0.1905	0.2571

TABLE 11  
*Increase in acidity in washed and in unwashed butter*

	AFTER 1 DAY	AFTER 2 DAYS	AFTER 3 DAYS	AFTER 4 DAYS	AFTER 7 DAYS	AFTER 11 DAYS	AFTER 32 DAYS	AFTER 64 DAYS
A, unwashed.....	0.0418	0.1221	0.3086					
C, washed.....	0.0388		0.0648			0.1038		
B, unwashed.....	0.0257	0.0514	0.1736					
D, washed.....	0.0151		0.0513			0.0841		
I, unwashed.....		(-0.0180)		0.0910	0.1160		0.2570	0.2573
II, washed.....		0.0770		0.1220	0.1540		0.2675	0.3341

This error can also be eliminated by calculating the final acidity in butter I and II in percentages of the final acidity of the corresponding free moisture:

*Acid formation in percentages of that in the free plasma*

Butter I, unwashed.....	82	53	57	57	72	72
Butter II, washed.....	59	48	50	43	56	64

In all cases the unwashed sample soured more than the washed butter.

This influence of washing can be shown still in another way. Table 9 shows that the unwashed butter up to the fourth day has already soured more than would be expected according to the



infected plasma. In the case of washed butter this occurs only after the eleventh day. Butter is protected very much against bacterial decomposition by washing.

The salted samples do not prove very much about the washing as in all cases the increase in acidity was very slight and as these differences fall mostly within the limits of error.

#### THE INFLUENCE OF WORKING

In most of our experiments normally worked butter is compared with overworked butter. The purpose of overworking was to get a finer moisture distribution but unfortunately this was not obtained in all cases because in some experiments (D, III, and V) water had been incorporated during the overworking process. This, of course, was present in form of larger drops which, however, did not consist of buttermilk but of water. Table 12 shows that in almost every case the overworked sample soured more slowly than did the normally worked check.

The lower part of the table shows the increase in acidity of the overworked butter in percentages of the acid increase of normal butter. These figures give us a good idea of the effect of working on protection of butter against bacterial decomposition. Only six out of the 34 figures lie above 100 and in these, the overworked butter soured faster than the normal samples. Of these six samples, four are within the limits of experimental error since only very small quantities of acid were present in one to two days old salted butter. Only samples II and III remain as exceptions; in these two samples, acid formation was about equal, especially towards the end of the experiment.

Apparently there is no relation between the inhibitive effect or overworking on bacterial action and the amount of plasma infected. The table even shows that in many cases the distribution of moisture droplets has become coarser through the incorporation of new water. The calculations, therefore, are wrong; for the newly incorporated water contained much less bacteria than did the original moisture in the butter. In the case of washed

butter, therefore, every calculation of infection shows too high figures.

As a result of these comparative experiments we may say that the keeping quality of the butter is increased by more working. The finer the distribution of the moisture droplets, the less and more slowly is butter decomposed by bacteria. Heavy working of butter not only results in a better control of the moisture content but also in the improvement of keeping quality from a bacteriological viewpoint.

The present experiments show a decrease of the acid production by working in a degree which cannot be accounted for by the finer distribution of the moisture droplets alone. If we assume that all water droplets are connected with each other, there is still another possible explanation. It may be assumed that by overworking the connections between the moisture droplets are broken up more and more, thus decreasing or even preventing diffusion. Experiment III shows a decrease in decomposition going somewhat parallel with the degree of working and the percentage of infected moisture, as is shown here:

*Final acidity in per cents of the acidity in the buttermilk, and per cents of infected moisture in the samples of experiment III*

	N (NORMALLY WORKED)	O (THOROUGH- LY WORKED)	C ("CREAMED")
Acidity after 60 days.....	41	32	12
Per cent of moisture infected.....	37.6	33.4	27.2

This may be explained by the assumption stated above, but an experiment showed that there was practically no difference in the time and speed of salt-dissolution in these three samples and that finally all moisture droplets were attracted by the salt. Another point may enter here which would also account for the greatly decreased acid formation. It is possible that during the working process some bacteria become entirely separated from all water, i.e. they may be distributed through the fat. These bacteria could not cause any further decomposition.

TABLE 12  
*Increase in acidity in normally worked butter and in over-worked butter*

NUM- BER	MOB- TILE CONTENT	PER CENT OF FLASK INFECTED	AFTER 1 DAY	AFTER 2 DAYS	AFTER 3 DAYS	AFTER 4 DAYS	AFTER 6 DAYS	AFTER 7 DAYS	AFTER 11 DAYS	AFTER 13 DAYS	AFTER 24 DAYS	AFTER 32 DAYS	AFTER 45 DAYS	AFTER 60 DAYS	AFTER 64 DAYS
A	12.6	26.4	0.0418	0.1221	0.3086										
B	13.1	17.0	0.0257	0.0514	0.1736										
C	12.2	23.0	0.0388		0.0648				0.1038						
D	13.2	30.0	0.0151		0.0513				0.0841						
E	11.3	4.8	0.0258		0.0007				0.0637						
F	12.7	32.3	0.0258		0.0782				0.0380						
G	10.9	7.0	0.0480		0.0100				0.0550						
H	12.4	22.0	0.0132		0.0134				0.0197						
II	12.4	29.2		0.0770		0.1220		0.1540				0.2675		0.3341	
III	15.0	41.0		0.0650		0.1035		0.1295				0.2695		0.3276	
IV	11.8	36.8		0.0200		0.0720		0.1000				0.0820		0.1035	
V	13.3	31.5		0.0271		0.0616		0.0616				0.0956		0.0771	
N	14.0	37.6		0.074			0.183			0.234	0.280		0.357	0.417	
O	14.0	33.4		0.016			0.093			0.106	0.141		0.177	0.331	
C	14.0	27.2		0.009			0.016			0.029	0.061		0.061	0.125	



## SALTING

No new knowledge is gained from the experiments with salted butter. They simply confirm that the salt, in the concentrations used, almost entirely inhibits acid formation.

Only for samples IV and V, a truly comparable free plasma had been obtained. (See table 6.) Here no acid formation was observed at all, but a very slight decrease in acidity. Whether this was of biological origin or not, was not determined. The corresponding butter samples showed a slight increase in titrable acidity but a decrease in hydrogen ion concentration. This behavior is difficult to explain. In the salted samples: E, F, G, H of the first experiment the increase in titrable acidity was smaller, the pH remained constant.

One might be tempted to compare the condition of the moisture in salted butter with that in washed butter and to assume that there are two different kinds of moisture droplets: Large, salt containing drops and small droplets more or less free from salt. But this is not very probable as Boysen (1) has shown by micro-motion pictures that the salt crystals attract all moisture droplets in their neighborhood. This attraction which always occurs in butter, but not in true solidified emulsions of skimmilk in butter-fat, is an important proof for the direct connection of all moisture droplets in butter. Yet 98 per cent of butter salt ordinarily are crystals under 0.75 mm. in diameter, and 30 to 60 per cent are under 0.5 mm. That means that there is one salt crystal to about three to five millions of water droplets in the case of normally salted butter. Some of the most distant droplets might escape the influence of salt, but the amount of this moisture is probably negligible and only the concentration of the salt in the larger drops would be increased a little. Furthermore, the salt crystals attract the moisture droplets only; the bacteria must remain at their old places, they become desiccated, so to speak.

However, the most effective part of salt-action is the concentration of the brine which amounts to the following percentages:

	SAMPLE E	SAMPLE F	SAMPLE G	SAMPLE H	SAMPLE IV	SAMPLE V
Per cent of salt.....	13.0	11.8	8.6	8.6	10.0	10.4

The relatively low salt concentration of 10 per cent is sufficient to almost entirely inhibit bacterial fermentation.

#### SUMMARY

1. The authors believe to have established the fact that a considerable portion of the moisture of cream becomes sterile in churning by being divided up into very small droplets. Even in sour cream butter there are more than 100 droplets to one bacterium. In this case about 40 per cent of the moisture, mostly the smaller drops, will become sterile; with pasteurized sweet cream butter more than 80 per cent of the moisture remain free from bacteria.

2. The amount of moisture shut off from bacteria depends upon two factors: i.e., the number of bacteria in the cream at the moment of churning and the degree of dispersion of the moisture in butter. In overworked butter more moisture is made sterile.

3. All experiments show uniformly that the formation of acid in the moisture within the butter is much slower than if the same amount of moisture is freed from fat and in the form of a continuous liquid. But it seems that the percentage of acid formed in butter slowly increases above the theoretical value computed from the amount of moisture which remains infected. This can be accounted for only by a diffusion of acid from the infected droplets to those free from bacteria. This diffusion is probable because the distance between the moisture droplets averages not more than five microns and diffusion of salt into unsalted butter has been established. Overworked butter shows less diffusion.

4. To prevent deterioration of butter by bacteria it seems advisable to churn pasteurized sweet cream with as few bacteria as possible and work it as much as possible without making it salty. Experiments by Guthrie of Cornell University (unpublished) demonstrated that of 10 churnings in 1926 and four churn-

ings in 1927 the highly worked butter scored higher after five months of cold storage than the butter which was worked but very little. However, the distinctly overworked butter scored no higher than the butter with very little working. This last effect might be due to air worked into the butter.

5. Washing decreases acid formation much more than would be expected from the fairly large amount of protein and lactose left in washed butter. This result can be accounted for by the assumption that washing does not change the concentration of the smaller droplets but dilutes the larger drops to almost pure water. As the large droplets are infected, while most of the smaller ones contain no bacteria, the separation between bacteria and food is much more complete than the chemical analysis of the butter will show.

6. These deductions and computations probably will not hold true for molds which have the ability to force their way from one droplet to the other.

#### REFERENCES

- (1) BOYSEN, HANS H. 1927 *Milchwirtschaftliche Forschungen*, Bd. 4, p. 221.
- (2) HITTCHER, K. 1918 *Landwirtschaftliche Jahrbücher*, Bd. 51, p. 489.
- (3) HUNZIKER, O. F. 1927 *The Butter Industry*, La Grange, Illinois, p. 273.
- (4) LORENZ 1901 *Dissertation*, Dorpat.
- (5) ORLA-JENSEN 1921 *Dairy Bacteriology*, Philadelphia, p. 121, footnote.
- (6) RAHN, O. 1926 *Milchwirtschaftliche Forschungen*, Bd. 3, p. 519.
- (7) RAHN UND SHARP 1928 *Physik der Milchwirtschaft*, Berlin.
- (8) SAYER, FARRAND, AND RAHN 1908 *Michigan Agr. Research Bull.* I.
- (9) SCHMIDT 1898 *Zeitschrift für Hygiene*, Bd. 28, p. 186.
- (10) TRICHERT, O. 1904 *Bakteriologisch-chemische Studien über die Butter in der Provinz Posen*, Jena.

# HEAT COAGULATION OF EVAPORATED MILK AS AFFECTED BY MIXING DIFFERENT GRADES OF RAW MILK\*

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Over the receiving platform of evaporated-milk factories there comes daily milk of varying quality and degree of purity. The purpose of this investigation has been to ascertain to what extent the heat coagulation of evaporated milk is affected by mixing a poor milk with a larger batch of good quality milk.

When a milk of low acidity is mixed with one of greater acidity a change in the hydrogen-ion concentration of the resulting mixture is observed. The hydrogen-ion concentration of a single sample is increased by bacterial action during aging. These changes in pH will, in turn, affect the heat stability of the milk. Rogers, Deysher and Evans (2) found no definite relationship between the hydrogen-ion concentration and the coagulation temperature of evaporated milks. Sommer and Hart (3) found that a slight increase in acidity increased the heat stability of some milks, while in others the stability was decreased. They attribute this variation in heat coagulation not directly to the hydrogen-ion concentration but to the effect of acidity upon the salt balance. Benton and Albery (1) found that outside the range pH 6.58 to 6.65, changes in pH produced a marked effect on heat stability. They ascribed the variations in stability observed within this range as due chiefly to changes in salt balance.

## EXPERIMENTAL

Skim milks of low heat stability were mixed with those of greater heat resisting capacities. The samples were mixed cold, forewarmed to 95°C. for ten minutes, evaporated under vacuum

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TABLE 1

*Effect of adding poor milk to good milk on the heat stability of evaporated milk*

LOT NUMBER	POOR MILK IN MIXTURE	ALCOHOL TEST	pH	TIME OF COAGULATION AT 130°C.
Series 1. Showing an initial increase, then a decrease in heat stability after the addition of poor milk				
	per cent			minutes
127	0	—	6.55	21
	8	—	6.55	26
	16	—	6.53	29
	100	+	6.35	0
201	0	—	6.64	12
	16	—	6.58	22
	32	—	6.53	25
	48	—	6.49	20
	100	±	6.47	3
203	0	—	6.63	21
	6	—	6.56	33
	12	—	6.54	33
	24	—	6.47	18
	36	—	6.42	3
	100	+	6.18	-0
207	0	—	6.54	24
	6	—	6.52	34
	12	—	6.50	38
	24	—	6.48	20
	36	±	6.43	14
	100	+	6.34	-0
403	0	—	6.62	16
	8	—	6.59	23
	16	—	6.57	27
	28	—	6.46	8
	44	±	6.40	4
	68	+	6.30	-0
Series 2. Showing a decrease in heat stability after the addition of poor milk				
324	0	—	6.64	19*
	6	—	6.62	15*
	12	—	6.59	17*
	24	—	6.51	10*
	40	±	6.46	7*
	60	+	6.40	5*

\* Coagulation at 115°C.

TABLE 1—*Continued*

LOT NUMBER	POOR MILK IN MIXTURE	ALCOHOL TEST	pH	TIME OF COAGULATION AT 120°C.
Series 2.— <i>Continued</i>				
	<i>per cent</i>			<i>minutes</i>
209	0	—	6.53	35
	6	—	6.50	28
	12	—	6.49	18
	24	±	6.48	7
	36	+	6.43	6
	100	+	6.25	—0
224	0	—	6.55	27
	12	—	6.55	27
	24	—	6.54	28
	48	—	6.51	25
	72	—	6.49	20
	100	—	6.46	19
223	0	—	6.64	27
	8	—	6.63	23
	16	—	6.61	21
	100	+	6.37	—0

to 18 per cent solids-not-fat, and sterilized at 120°C. in tins until coagulation occurred. Samples were taken before forewarming, on which the pH and the alcohol test were determined.

The good quality milk used was separated skim milk, usually fresh on the morning of the experiment. The poor milk was separated skim, allowed to stand either at room temperature or in ice box until it was alcohol positive or nearly sour. Milk was considered alcohol + when a precipitate occurred with equal parts of milk and 74 per cent alcohol. The ± point was taken as + to 74 per cent and — to 68 per cent alcohol. When — to 68 per cent alcohol the milk was considered alcohol negative. The poor milk added to that of good quality is calculated as a given percentage by weight of the total mixture when made up to the total with the good sample before concentration.

Milks of two different types were found, namely, those whose stability was first increased by addition of poor milk, and those whose stability was decreased under the same conditions. The

milks used were from the United States Experiment Farm at Beltsville, Maryland, and from two local dairies. The milk from each source was consistent in its behavior from day to day.

In table 1, series 1 are shown some data from the experiments on milk from the Beltsville farm which could always be improved during this work by addition of poor milk. Series 2 of the table contains data obtained from the milks of the two local dairies

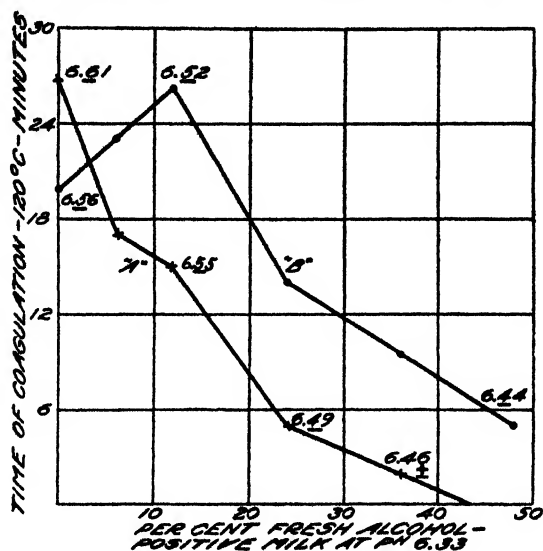


FIG. 1. SHOWING THE EFFECT OF SMALL ADDITIONS OF FRESH ALCOHOL-POSITIVE MILK UPON THE HEAT COAGULATION OF TWO DIFFERENT TYPES OF MILK

The pH and alcohol reactions are indicated

which decreased in stability upon addition of poor milk. Additions of poor milk in any proportion to this type of product were always detrimental in their influence on the heat coagulation of the resulting mixture.

If these milks were not at their optimum pH for maximum heat resistance, then addition of lactic acid should have an effect similar to that of high acid milk developed by bacterial action. Benton and Albery (1) were able to increase greatly the stability of certain samples of fresh milk by addition of various amounts

of lactic acid. Similarly additions of lactic acid to the two types of milks shown in table 1 affected their heat stabilities in the same way as did mixing with aged milk.

It has often been observed that a few cows can be found in many herds whose milk is either continually or occasionally alcohol positive in reaction at the time of milking. The effect of additions of such fresh alcohol-positive milk from three cows habitually giving this type of milk, to fresh normal milks, is

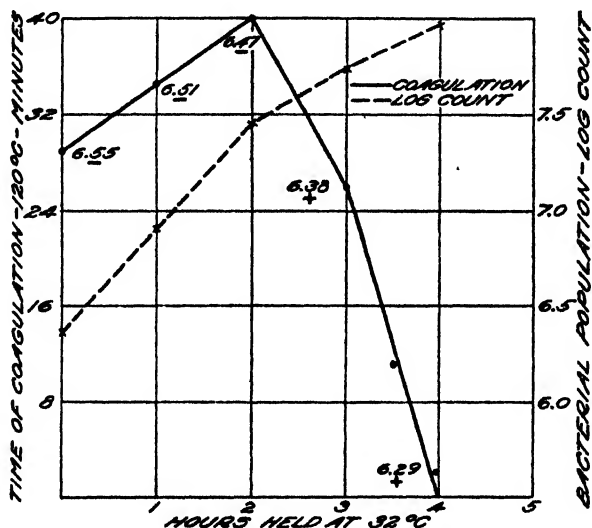


FIG. 2. SHOWING A MILK THE HEAT STABILITY OF WHICH IS FIRST INCREASED THEN DECREASED BY AGING

The pH and alcohol reactions are indicated

shown in figure 1. The pH and the reaction to the alcohol test are shown on the figure for each milk.

Curve A is milk from one of the local dairies, while curve B is for milk from the Beltsville farm. The stability of these samples when mixed with varying percentages of the same fresh, alcohol positive milk is similar to their heat stability when mixed with milks whose quality has been lowered by aging and by increase in acidity. The trend of curve A is typical of the behavior

of the milk in series 2 of table 1. Curve B shows the manner in which the milk reported in Series 1 would plot graphically.

Mixing a poor quality milk with one of better quality should have the same effect upon heat stability as aging a milk of high stability in place of adding poor milk. The pH of the resulting product is changed in both cases by bacterial action.

Samples of milk were aged at 30° to 34°C. until they became alcohol positive.<sup>1</sup> At various intervals samples were taken for

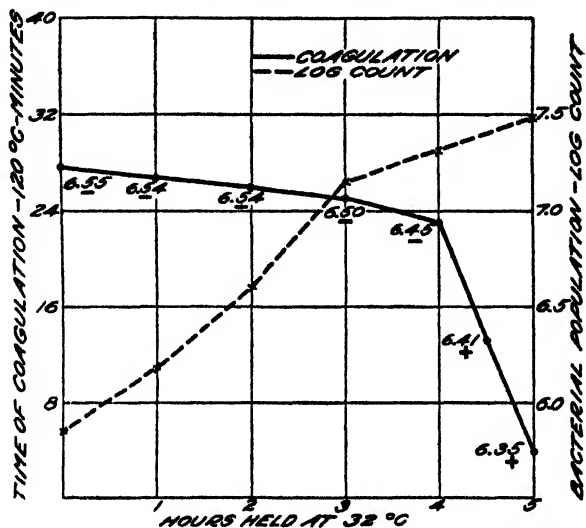


FIG. 3. SHOWING A MILK, THE HEAT STABILITY OF WHICH IS DECREASED BY AGING

The pH and alcohol reactions are indicated

evaporation and coagulation and for bacterial plating and determination of pH. No consistent relationship was observed between the pH changes or the coagulation time and the type of bacterial growth. Colonies were isolated but no outstanding change could be considered a result of the action of an individual organism.

In figure 2 are plotted the data obtained for one of the milks

<sup>1</sup> Data on aged milk is from unpublished work of Anne G. Benton and the author.

which was first increased then decreased in heat stability during aging. The bacterial population has been expressed as the log count of the colonies found.

Figure 3 shows the results for one of the milks, the heat stability of which was decreased by aging.

#### DISCUSSION

The result of adding small percentages of poor milk to a milk of good quality can be expected either to increase or decrease the initial heat stability of the good sample. No method is available by which the result of such mixing may be foretold. The conclusions of Benton and Albery that each sample of milk, whether mixed or from a single cow, presents a separate colloidal system, appears to apply here. There apparently is an optimum pH for each system, at which optimum the greatest heat stability is obtained.

The nature of the results is the same, whether good and poor milks are mixed, or whether a single milk is aged and the pH developed by bacteria. In both cases the milk was originally at the pH suitable for its greatest stability or it attained that pH during treatment. It has been noticed during this investigation that the periods during which the milk was not normally at its maximum stability were often a week or longer in length.

It is probable that mixed herd milk is usually at its optimum pH originally but that there are sometimes short periods of time when the milk after secretion is not balanced correctly to give it maximum heat stability. At these times adjustment of the pH would be beneficial.

Aging or incubation of the product was always accompanied by an increase in bacterial growth as well as a decrease in pH.

The stability always changed with an increase or decrease in pH.

#### SUMMARY

When small amounts of poor milk were mixed with larger batches of good quality milk an evaporated product of uncertain heat stability was produced. The change in heat stability which

was observed as a result of mixing quality, of adding lactic acid, or of aging the milk is ascribed to the accompanying change in pH. It is again suggested that for every milk there is an optimum pH, the attainment of which will result in maximum heat stability for the system involved. Identical results in terms of heat coagulation may be obtained by mixing milks of different quality or by aging a single sample.

#### REFERENCES

- (1) BENTON, A. G., AND ALBERY, H. G.: Studies on the stability of evaporated milk during sterilization, with reference to the hydrogen ion concentration, alcohol test, and the addition of specific buffers. *Jour. Biol. Chem.*, lxxviii, (1926) 251.
- (2) ROGERS, L. A., DEYSHER, E. F., AND EVANS, F. R.: The relation of acidity to the coagulating temperature of evaporated milk. *Jour. Dairy Science*, iv, (1921) 294.
- (3) SOMMER, H. H., AND HART, E. B.: Heat Coagulation of Evaporated Milk, Research Bulletin 67, Wis. Agr. Expt. Station (1926).

# THE RELATION OF SOYBEAN HAY AND GROUND SOYBEANS TO FLAVOR AND COMPOSITION OF MILK AND BUTTER\*

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Several reports which reached the Department of Dairy Husbandry during the past year stated that rations containing soybean hay or ground soybeans caused undesirable flavors in milk, cream, and butter; these products being affected to such an extent that they were not marketable or that their value was lowered.

Previous investigations (1), (2) have shown that the feeding of soybeans, soybean oil meal, and soybean oil cause the body of butter to be noticeably soft, but so far as the authors can learn no investigations have been reported in which harmful flavors resulted from rations of this character. In fact, Lindsey et al. (2) report that soybean oil meal imparted no particular flavor to butter.

Experiments were therefore undertaken with the object of determining the effect of rations containing soybean hay and ground soybeans upon the flavor and composition of milk, cream, and butter.

Twenty-four cows each producing daily 15 to 30 pounds of milk were divided into three groups. Division was made on the basis of breed, age, live weight, days from last calving, stage of gestation, and milk yield. The average daily production of each group at the beginning of the experiment was approximately 20 pounds per cow. On account of advancing lactation in one case and indigestion in another, it was necessary to omit the records of two cows during the last week of the experiment. The experimental periods were each one week in length.

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## EXPERIMENTAL RATIONS AND PROCEDURE AT BARN

During the first week, all groups were fed the control ration consisting of red clover hay and "herd mixture" (300 pounds ground corn, 400 pounds ground oats, 200 pounds wheat bran, 100 pounds linseed oil meal, 15 pounds bonemeal, 15 pounds salt). Group A was continued on this ration throughout the experiment.

During periods 2, 3, 4, and 5, group B was fed soybean hay and a concentrate mixture. The soybean hay fed during periods 2, 3, and 4 was of high grade, having been cut when the beans were in the early stages of development. It had a high green color and was very palatable. The leaves formed 45 per cent of the weight of the hay and the pods and beans 17 per cent. Seven per cent of weeds, mostly foxtail, was present.

It seemed desirable to learn the effect of poor quality soybean hay also. Accordingly, during period 5, some badly weathered soybean hay having many large, moldy beans and pods was fed. The leaves formed 21 per cent, the beans 21 per cent, and the pods 22 per cent of the weight of the hay. Four of the cows refused this hay, so their records are omitted from this period. The grain mixture fed group B during period 2 was the herd mixture. This was replaced during periods 3, 4 and 5 by grain mixtures containing 10, 15, and 20 per cent of ground soybeans, the same mixtures as fed to group C during periods 2, 3, and 4.

Group C was fed the same kind of hay as group A. During periods 2, 3, 4, and 5, the grain mixtures consisted of ground corn, ground oats, and wheat bran, together with enough ground soybeans to form approximately 10, 15, 20, and 25 per cent of the mixtures in the respective periods. Bonemeal and salt were added at the rate of about 1.5 per cent each.

During period 6, both groups B and C were returned to the control ration of clover hay and herd mixture. No silage was fed during the experiment. All the experimental periods were consecutive, changes in rations being made abruptly.

A condensed summary of the amounts of feed consumed, together with the production records of the different groups, are shown in table 1. Hay consumption was large, ranging from 20

to 25 pounds per cow daily in most of the periods. Concentrates were fed at the rate of about 1 to 2½ pounds of milk, the maximum

TABLE 1

*Production and feed records of cows during study of the effect of soybeans on the flavor and composition of milk and butter*

Results expressed in terms of weekly averages per cow

WEEK	RATION	MILK	FAT	FAT	F. C. M.†	LIVE WEIGHT	FEED EATEN	
							Hay	Grain

Group A								
			per cent	pounds	pounds	pounds	pounds	pounds
1	Clover hay—herd mixture	127.9	3.90	4.99	126.01	1,134	140.3	58.9
2	Clover hay—herd mixture	121.5	3.89	4.72	119.40	1,127	150.7	55.4
3	Clover hay—herd mixture	118.3	3.88	4.59	116.17	1,132	157.4	49.0
4	Clover hay—herd mixture	103.5	3.88	4.02	101.70	1,131	153.2	46.4
5†	Clover hay—herd mixture	93.5	4.07	3.80	94.40	1,116	161.7	44.0

Group B								
			per cent	pounds	pounds	pounds	pounds	pounds
1	Clover hay—herd mixture	140.2	3.85	5.40	137.08	1,192	137.0	60.0
2	Soybean hay—herd mixture	142.7	3.84	5.48	139.28	1,163	124.0	60.9
3	Soybean hay—mixture no. 1*	134.3	3.87	5.20	131.72	1,155	148.0	55.7
4	Soybean hay—mixture " 2*	133.1	3.95	5.26	132.14	1,158	156.1	56.0
5‡	Soybean hay—mixture " "§	144.6	3.77	5.45	139.59	1,072	159.5	61.3
6†	Clover hay—herd mixture	126.2	3.78	4.78	122.18	1,113	154.7	56.0

Group C								
			per cent	pounds	pounds	pounds	pounds	pounds
1	Clover hay—herd mixture	141.3	4.16	5.87	144.57	1,152	148.2	60.0
2	Clover hay—mixture no. 1*	143.4	4.01	5.75	143.61	1,135	163.7	60.1
3	Clover hay—mixture " 2*	144.2	4.12	5.95	146.93	1,145	166.4	61.3
4	Clover hay—mixture " 3*	138.5	4.14	5.74	141.50	1,136	156.3	57.4
5	Clover hay—mixture " 4*	136.4	4.03	5.50	137.06	1,158	172.5	56.9
6	Clover hay—herd mixture	135.0	4.02	5.43	135.45	1,152	172.0	56.0

\* Mixture no. 1 contained 10 per cent ground soybeans; no. 2, 15 per cent; no. 3, 20 per cent; no. 4, 25 per cent.

† F. C. M. (fat corrected milk) equals  $.4 \times \text{milk} + 15 \times \text{fat}$ . (Ill. Agr. Exp. Sta. Bul. 245).

‡ Seven cows only.

§ Four cows only.

amount of soybeans fed being about 2½ pounds per head daily. Barn records of milk yield and weekly composite samples taken

from each milking served as a basis for the computation of production records. The samples were tested for butterfat by the Babcock method. The butterfat tests shown in this table thus differ slightly from the averages of the butterfat contents of the mixed milk as determined by the Mojonnier method.

Care was taken at the barn to keep the milk as clean and free from odors as possible. Cans and strainers were provided for the milk of each group. These cans were distinguished by covering the shoulders with paint of different colors, red representing the control ration, yellow the soybean hay ration, and blue the ground soybeans ration. The same color scheme was followed on the feed sheets, milk records, grain mixtures, and cards over the mangers.

The milk was taken daily to the Dairy Manufactures Building, where tests were carried out to determine the effect of the rations upon the flavor and composition of milk and butter.

#### PROCEDURE AT DAIRY MANUFACTURES BUILDING

The milk was first clarified. (During the first week, the milk was filtered through ordinary milk strainers, but this procedure was found impractical due to the time involved.) Samples of the raw milk were taken for cream volume studies and judging. The milk was then pasteurized by heating to 142°F. and holding for thirty minutes at that temperature. A 50-gallon starter can type of vat was used for this purpose. A portion of the milk was then pumped over a surface cooler. The remaining milk was pumped into a power separator and separated at about 120°F. The skim milk and cream were then pumped over the surface cooler and sampled.

Portions of the raw, and the cooled pasteurized whole milk were placed in 100 cc. graduates in a 40°F. refrigerated room for the purpose of cream volume measurement. These tests were run in duplicate and were read approximately twenty-four hours after setting.

The raw milk was tested daily for fat and total solids using the Mojonnier Milk Solids Tester. Acidity determinations were also made on the raw milk using N/10 NaOH and phenolphthalein as an indicator.

The raw and pasteurized whole milk, the pasteurized skim milk and cream were judged daily for odor and flavor.

The pasteurized cream was held at 40°F. in 10-gallon milk cans and at the end of seven days the accumulated cream was churned. The cream was standardized to 35 per cent fat with fresh pasteurized skim milk and 10 per cent of starter added. A Cherry Junior Churn (No. 2B) with a capacity of about 30 gallons of cream was used. The churning procedure followed was the standard procedure for the creamery.

The butter was scored one day after being made and again about one week later. From seven to eight individuals sampled the butter. The score on the butter was placed by H. A. Ruehe and P. H. Tracy of the Dairy Manufactures Division.

Each lot of butter was analyzed for fat, moisture, salt, and curd using the modified Kohmann method. In addition the Iodine Number (Hanus) and the Refractive Index of each lot was determined. An attempt was made to determine the melting point of the butter but reliable checks could not be obtained.

#### SOYBEANS HAD NO EFFECT ON MILK FLAVOR

Samples of the milk before and after it was pasteurized as well as the cream and skim milk resulting from separating the pasteurized milk were judged daily for flavor. In no case was it possible to detect any flavor that might be attributed to either the ground soybeans or soybean hay. The flavor of the milk varied. In some cases the control lot was the best. In other cases the milk from the soybean groups was the better. In several instances the three lots were equally good. In practically all cases where a defect in flavor occurred it could be attributed to factors other than feed such as a cowy flavor or barn odors. It was noticed that the milk from group C, during the interval this group was receiving the maximum amount of ground soybeans, had a flavor different from the other groups, but it was not an objectionable flavor. However, this flavor which was rather noticeable at the beginning of the period was hardly detectable in the milk received during the last two days of the week. On the whole, the milk of groups B and C was fully as good, if not better, in flavor as that of group A.

## COMPOSITION OF MILK AFFECTED SLIGHTLY

The data are not comprehensive enough to draw definite conclusions as to the effect of either ground soybeans or soybean hay upon the fat content of the milk. The data, given in table 2, indicate that after groups B and C had been returned to the control ration in period 6, that the omission of soybeans from the ration seemed to cause a slight lowering of the fat content of the milk.

TABLE 2  
*Composition of milk as affected by soybean rations\**

WEEK	FAT IN MILK OF GROUP			SKIM MILK SOLIDS OF MILK OF GROUP			ACIDITY OF MILK OF GROUP			CREAM VOLUME OF MILK OF GROUP					
										A		B		C	
	A	B	C	A	B	C	A	B	C	Raw	Pasteur- ised	Raw	Pasteur- ised	Raw	Pasteur- ised
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent						
1	4.09			9.06			0.152			14.6	11.6				
2	4.12	3.99	4.22	8.82	8.83	9.17	0.152	0.155	0.152	13.6	11.9	12.3	11.3	15.3	13.9
3	3.98	4.07	4.36	8.91	8.88	9.06	0.154	0.153	0.153	12.9	11.1	12.9	11.4	15.7	14.7
4	4.05	4.09	4.25	8.88	8.92	9.09	0.153	0.153	0.154	12.9	11.8	12.1	11.6	14.9	14.4
5	4.27	4.34	4.29	9.01	8.93	8.97	0.153	0.151	0.154	14.4	12.1	13.0	11.9	15.4	14.2
6		4.08	4.08		8.95	9.07						14.1		16.4	

\* Computed from weighted averages for entire period.

The soybean rations had no effect upon the acid content of the milk as measured by titration with N/10 NaOH.

## DIFFERENCES FOUND IN QUALITY OF BUTTER

None of those sampling the butter were able to detect any flavor defect that could be attributed to the soybeans. In several instances the flavor of the butter from the groups fed soybeans was better than that of the control. All of the butter produced while clover hay was being fed, with the exception of period 2, had what was designated as an unclean flavor. In only one lot of butter, that from the soybean hay ration of period

4, was an unclean flavor found, and it was not so pronounced as that of the butter from the control ration during the same period.

There was a noticeable effect of soybeans upon the body of the butter. The butter from the soybean groups seemed to have

TABLE 3  
*Composition and score of butter as affected by soybean rations*

WEEK	FAT IN BUTTER	IODINE NUMBER	REFRACTIVE INDEX	SCORE OF FLAVOR		SCORE OF BODY		TOTAL SCORE	
				(1)	(2)	(1)	(2)	(1)	(2)
Group A. Control ration									
	<i>per cent</i>								
1	83.9	40.05	1.4547	36.5	36.0	24.5	24.5	91.0	91.0
2	82.6	40.62	1.4547	37.0	37.5	24.5	24.5	91.5	92.0
3	83.1	40.50	1.4548	36.0	36.5	24.0	24.0	90.0	90.5
4	82.3	41.66	1.4548	36.0	36.5	25.0	25.0	91.0	91.5
5	82.0	40.57	1.4548	35.0	33.0	24.0	24.0	89.0	87.0
Group B. Soybean hay ration									
2	82.9	40.02	1.4547	37.0	37.5	24.0	24.0	91.0	91.5
3	83.6	39.46	1.4547	37.5	37.5	24.0	24.0	91.5	91.5
4	83.1	40.09	1.4548	37.0	36.5	24.0	24.0	91.0	90.5
5	83.3	43.13	1.4554	35.0	35.0	23.0	23.0	88.0	88.0
6*	82.5	44.02	1.4554	36.0	35.0	24.3	24.3	90.3	89.3
Group C. Ground soybeans ration									
2	82.6	39.64	1.4546	37.0	37.5	23.5	23.5	90.5	91.0
3	80.1	40.55	1.4547	37.5	37.5	23.5	23.0	91.0	90.5
4	82.2	41.18	1.4547	37.5	37.0	24.0	23.5	91.5	90.5
5	82.5	41.80	1.4550	35.5	35.5	23.0	22.5	88.5	88.0
6*	83.1	40.90	1.4548	36.0	35.0	24.5	24.5	90.5	89.5

\* Control ration fed.

(1) Scored day after churning

(2) Scored one week after churning.

more of a gummy consistency than the other (this butter was judged at a temperature of about 40°F.). The ground soybeans was more of a factor in contributing to this condition than the soybean hay. This defect was not serious, although as shown in table 3, the score of the body of the butter from the soybean

rations was usually from one-half to one and one-half points lower than that of the butter from the control ration.

The results obtained in the butter constants determinations are not conclusive. It was impossible to obtain satisfactory melting points. The refractive index values were fairly uniform though there was a tendency for the iodine number to increase with the increased soybean content of the ration. This increase was more consistent in the case of the ground soybeans ration than in the case of the soybean hay ration.

#### CREAM VOLUME OF MILK IS HIGHLY VARIABLE

An interesting phase of this study was that of cream volume. There was a marked tendency for volume of cream in the group C milk samples to exceed that in the milk from the other groups. This relationship seemed to hold true even when the fat content of the group C milk was equal to or less than that of the other milk.

Determinations of cream volume of the milk of the individual cows of groups B and C were made during period 6, after both groups had been returned to the control ration. The results indicate that the variation in cream volume was not due to the effect of feed. The explanation for the higher cream volume of the milk of group C seems to be that this group included individual cows yielding milk having a greater cream volume than the milk of the cows of group B.

#### SUMMARY

Three groups of eight cows each were fed during six consecutive weekly periods on rations consisting of either red clover hay or soybean hay, and a concentrate mixture with or without ground soybeans. The ground soybeans, when fed, formed from 10 to 25 per cent of the mixtures.

The milk produced was used in studies of flavor and composition, and butter was made from the cream.

Neither high quality, nor poor quality, moldy soybean hay was found to have any effect upon the flavor of the milk (raw or

pasteurized), skim milk, cream, or butter. Ground soybeans were likewise without effect on flavor. The fat content of the milk was affected but slightly, and the acidity was not changed appreciably. Marked differences in cream volume were noted but these seemed to be associated with individual cows.

The most pronounced effects of the rations were upon the body of the butter. Ground soybeans caused the body to be gummy and the condition became worse when the proportion of soybeans in the ration was increased. Soybean hay had a similar effect but to a less degree. There was a tendency for the iodine number to increase slightly with the larger amounts of soybeans in the ration.

#### REFERENCES

- (1) OTIS, D. H. 1904. Experiments with Dairy Cows. Kansas Agric. Exp. Sta., Bull. 125.
- (2) LINDSEY, J. B., HOLLAND, E. B., AND SMITH, P. H. 1909. Effect of Soy Bean Meal and Soy Bean Oil upon the Composition of Milk and Butter Fat, and upon the Consistency or Body of Butter. Mass. Agric. Exp. Sta. Twenty-first Annual Report, Part II, page 66-110.



# COTTONSEED MEAL AS A FEED FOR DAIRY CALVES\*

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Cottonseed meal is seldom recommended as a feed for calves, and only limited amounts are recommended for lactating dairy cows. This is due to a common opinion that cottonseed meal contains a poisonous principle which produces cottonseed meal injury (1). During the last five years, 20 Holstein heifers were grown from six to twenty-four months of age at Michigan State College on a grain ration consisting of cottonseed meal as the principal source of protein. The animals also received an ample supply of timothy hay and corn silage. No physical defects were produced although 2 pounds of cottonseed meal were fed to these animals daily throughout this period. The present paper is a preliminary report covering the effects of cottonseed meal feeding on the health of growing dairy heifers. The effects on reproduction and lactation will be reported later.

## REVIEW OF LITERATURE

Although Nevens (2), Aberhalden and Rostoski (3), Richardson and Green (4), Mendel (5), Osborne and Mendel (6), and McCollum and Simmonds (7) have demonstrated the high biological value of cottonseed protein, several investigators have reported injurious effects from feeding cottonseed meal to cattle. Dinwiddie and Short (8) state the symptoms of cottonseed meal poisoning in cattle are recognized by a reeling unsteady gait, which appears to be due in part to muscular incoördination and, in part, in the later stages, to partial or complete blindness. These workers produced cottonseed meal injury in two Jersey steers. One animal which received 1 pound of cottonseed meal daily for each 100 pounds of live weight, with cottonseed hulls for roughage

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showed the symptoms of cottonseed meal poisoning at the end of 70 days. This animal was anemic, having an erythrocyte count of only 3,400,000 and a hemoglobin of 50 per cent. The other steer did not develop the symptoms of cottonseed meal injury until after 116 days of cottonseed meal feeding. Jones, Lush, and Jones (9) found a lessening of appetite with a consequent decrease in the rate of gains as the preliminary symptoms of cottonseed meal injury in fattening steers.

In order to determine the effect of cottonseed meal upon growth and reproduction of cows, Combs and Curtis (10) placed five heifers on each of the following rations:

*Ration 1.* Cottonseed meal (1 pound daily per 100 pounds live weight). Cottonseed hulls ad libitum.

*Ration 2.* Cottonseed meal  $\frac{1}{2}$ , crushed corn  $\frac{1}{2}$  (1 pound daily per 100 pounds live weight). Cottonseed hulls ad libitum.

*Ration 3.* Cottonseed meal (1 pound daily per 100 pounds live weight). Cottonseed hulls  $\frac{1}{2}$  and corn silage  $\frac{1}{2}$ , ad libitum.

*Ration 4.* Cottonseed meal (1 pound daily per 100 pounds live weight), copper sulphate solution. Cottonseed hulls ad libitum.

*Ration 5.* Cottonseed meal (1 pound daily per 100 pounds live weight). Cottonseed hulls  $\frac{1}{2}$  and beet pulp  $\frac{1}{2}$ , ad libitum.

Some of the heifers became weak, had convulsions, and became blind. All of the heifers in lot 1 died, as did some of the animals in the other lots. Those that lived became weak and were becoming blind so they were removed from the experiment.

McNutt (11) reported that a bull calf fed on skim milk and cottonseed meal died after 71 days on this ration. One-fourth pound of cottonseed meal was fed per day at first, but this amount was gradually increased to  $1\frac{1}{2}$  pounds per day. In another experiment, McNutt fed one group of heifers a basal ration of corn, oats, and wheat bran, equal parts by weight, and hay, and corn silage. Another group were fed cottonseed meal, corn, oats, hay, and silage. The four younger heifers in the latter group either failed to gain or lost considerable weight during the first two months on this ration. They also appeared very unthrifty. When placed on the basal ration they made very satisfactory

gains. According to Kennedy and Marshall (12), three head of cattle in a lot of 20 died after receiving a ration consisting of 25 pounds of corn and cob meal,  $2\frac{1}{2}$  pounds cottonseed meal, and wheat straw for roughage for 42 days. The rest of the animals became blind.

Many theories have been advanced attempting to explain the cause of cottonseed meal injury. Only a few are enumerated in this report. Crawford (13), working with rabbits concluded that the chief ptomaine principle in cottonseed meal was a salt of pyrophosphoric acid. However, Withers and Ray (14) were unable to substantiate Crawford's conclusions. Forbes, Beegle, and Mensching (15) found the excess of acid over base forming elements in 100 grams of cottonseed meal was equal to 7.7 cc. of normal acid. Wells and Ewing (16) reported that the excess of acid over base forming elements was 8.21 cc. of normal acid. They then thought that cottonseed meal injury might be due to an acidosis. Later, in an extensive investigation dealing with swine, they were unable to produce evidence to support this hypothesis. Rommel and Vedder (17) found that symptoms of injury produced when pigs were fed a ration of nine parts steamed polished rice and one part tankage were quite similar to the symptoms produced in other pigs which were fed a ration of two parts corn and one part cottonseed meal. These men then concluded that beri-beri and cottonseed meal injury were analogous diseases. However, it is unlikely that cottonseed meal injury in cattle is analogous to beri-beri since Bechdel, Eckles, and Palmer (18) showed that the vitamin B requirement of calves is either extremely low or that it is synthesized in the animal body. McGowan and Crichton (19) stated that the hemoglobin of pigs suffering from cottonseed meal injury was sometimes as low as 20 per cent although it was generally about 40 per cent. This condition was relieved by feeding 40 grams of ferric oxide daily to the mothers of the suckling pigs. The investigators concluded that the principal factor causing cottonseed meal injury was a lack of sufficient iron in the ration. Withers and Carruth (20) reduced the injury in swine which were fed cottonseed meal and corn by adding either copperas or ferric chloride to the ration.

Withers and Brewster (21) found that certain iron salts tended to alleviate the toxicity produced in rabbits by the feeding of cottonseed meal.

Withers and Carruth (22) were the first investigators to attribute the injury produced by cottonseed meal to gossypol. They have shown (23) that gossypol is toxic when fed to rats, rabbits, guinea pigs, or swine.

The theory that gossypol is responsible for cottonseed meal injury was further substantiated by Schwartz and Alsberg (24) who found that diets containing 0.225 per cent of gossypol killed rats within three days.

Sherwood (25) found that gossypol in 14 samples of cottonseed meal, prepared from kernels cooked in open kettles, varied from 0.021 to 0.150 per cent and the d-gossypol content from 0.544 to 0.963 per cent. Twenty-two samples of meal from mills using the continuous cooker process varied from 0.007 to 0.228 per cent of gossypol and from 0.633 to 1.076 per cent of d-gossypol. Four samples of meal made by the expeller process varied from 0.02 to 0.102 per cent of gossypol and from 0.335 to 0.505 per cent of d-gossypol.

Menaul (26) produced death in a four pound rabbit in about four minutes by injecting 0.1 gram of gossypol into the marginal vein of the ear. A half gram administered orally to another rabbit produced no abnormal symptoms. Another rabbit injected intraperitoneally with 0.5 gram died on the fourth day. Continued feeding of 0.1 gram of gossypol per day to each of four rabbits caused intestinal inflammation resulting in death in about 14 days. He found the gossypol tended to inhibit the liberation of oxygen from oxyhemoglobin. Only 46.8 per cent as much oxygen was liberated from 2 cc. of erythrocytes in 1 cc. of physiological salt solution having 0.02 gram of gossypol, as from the controls.

Withers and Carruth (23) maintain that, when cottonseeds are heated in the presence of water, gossypol is converted to less soluble d-gossypol, which is also less toxic. They account for the variation in toxicity by the variation of gossypol and moisture in the cottonseed from which the meal is made, and by the variation

in heating during the manufacturing process. The works of Sherwood (25), Dowell and Menaul (27), Gallup (28), Edgerton and Morris (29) tend to support this view. Osborne and Mendel (30) found cottonseed meal non toxic, but cottonseed kernels that contained about 0.6 per cent of gossypol were toxic to rats. Nevens (31) and Richardson and Green (4) also showed that cottonseed meal and cottonseed flour are not toxic to rats. There is no evidence that the injury produced in cattle which are fed cottonseed meal is due to gossypol.

Several investigators have reported that the injurious effects of cottonseed meal are at least partially neutralized by feeding other feeds with the cottonseed meal.

Wright (32) fed 2000 head of cattle a daily ration of 8 pounds of cottonseed meal and 25 pounds of cottonseed hulls, with a feed of hay once a week throughout the fattening period. These cattle (mostly four to six-year-old steers) made an average gain of 75 pounds each per month. The only abnormality observed was an occasional diarrhea, which was relieved by feeding hay for a few days. Stanley (33) concluded from results secured in steer feeding work that, if silage replaced cottonseed hulls as roughage, cottonseed meal might be fed for a longer time without ill effects. Burtis (34) concluded that some other grain and hay should be fed with cottonseed meal to fattening steers. Michels (35) reports, "Our feeding trials have well established the fact that cottonseed meal can be fed with much greater safety in conjunction with silage than with dry roughage." Michels did not state whether the dry roughage was hay, straw, or cottonseed hulls.

The North Carolina Station (36) reported that a herd of 20 cows fed heavily on cottonseed meal were apparently normal when kept in a four acre pasture. When this herd was moved to a dry lot, abortion, dead or underweight calves, and living blind calves were obtained. The cows also gradually lost weight, became stiff, and had edema. In some cases these animals had severe fits from which they ultimately died. Other cows had fits occasionally and became partially blind and some permanently blind. These results suggested nutritive deficiencies in rations consisting of cottonseed meal and hulls. Such rations supplemented by vitamins A and B and calcium gave good results.

## EXPERIMENTAL

*Part I*

The similarity of the symptoms of cottonseed meal injury in cattle to those produced when concentrates alone were fed suggested the possibility that both conditions might be a result of the same deficiency. In order to study this relationship, two lots of two calves each were used. The animals in lot 1, C 43 and C 47, received cottonseed meal as the principal source of protein while those in lot 2, C 44 and C 45, received corn gluten meal and corn distillers grain. Both groups received corn and oats and wheat

TABLE 1  
*Feed record of calves fed cottonseed meal with wheat straw as a roughage*  
Lot 1—Part I

30-DAY PERIODS	C 43					C 47				
	Skim milk	Cotton-seed meal	Yellow corn	Oats	Wheat straw	Skim milk	Cotton-seed meal	Yellow corn	Oats	Wheat straw
	pounds	pounds	pounds	pounds	pounds	pounds	pounds	pounds	pounds	pounds
1	360	66	46	46	Ad. lib.	362	43	46	46	Ad. lib.
2	308	74	70	70	Ad. lib.	420	60	60	60	Ad. lib.
3	785	160	29	29	Ad. lib.	420	91	45	45	Ad. lib.
4	15	168	29	29	Ad. lib.		112	45	45	Ad. lib.
5		180	30	30	Ad. lib.		52	27	27	48
6		180	33	33	Ad. lib.		82	43	43	57
7		153	45	45	Ad. lib.		14	8	8	9†
8		159	45	45	78					
9		60	30	30	54*					

\* Twenty days.

† Eight days.

straw ad libitum. Salt was placed before the animals at all times. Skim milk was fed to 180 days of age. The animals used were four bull calves, three Holsteins and one Jersey, which had received an adequate ration up to the time they were placed on experiment which was about 90 days of age. The amount of feed consumed by 30 days periods is recorded in table 1. Growth of the calves is found in figures 1 and 2.

Figures 1 and 2 show that the gain in body weight of all four

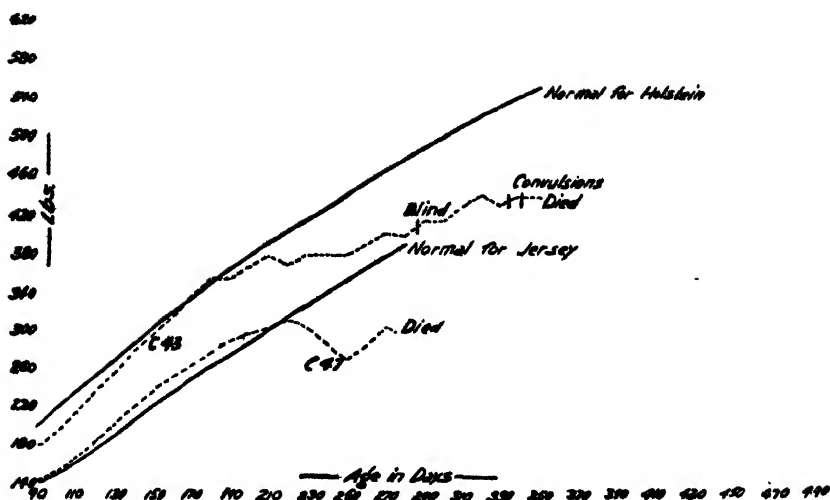


FIG. 1. GROWTH IN WEIGHT OF CALVES RECEIVING COTTONSEED MEAL AS THE CHIEF SOURCE OF PROTEIN WITH WHEAT STRAW AS A ROUGHAGE

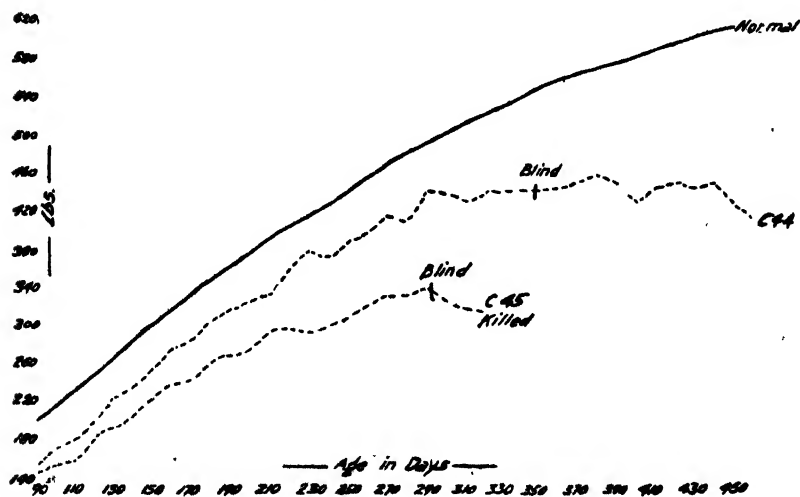


FIG. 2. GROWTH IN WEIGHT OF CALVES RECEIVING CORN GLUTEN MEAL AND CORN DISTILLERS GRAIN AS THE PRINCIPAL SOURCE OF PROTEIN WITH WHEAT STRAW AS A ROUGHAGE

animals was fairly good for a time. The rate of gain was more rapid and the ensuing decline greater in case of the 2 animals receiving cottonseed meal than where corn gluten and corn distillers were fed. This was probably due to the greater palatability of the cottonseed meal which caused an increased intake of concentrates by calves C 43 and C 47. Stiffness and swelling around the hocks were observed in all four animals. C 43 had a convulsion that lasted about ten minutes on the 287th day of age. Seven days later another convulsion of short duration was observed. Diarrhea was also manifested from time to time. This animal was found dead on the 350th day of age. It probably died in convulsions. C 47 died at 275 days of age. This animal did not become blind, nor manifest tetany, although the heavy feeding of concentrates injured its health.

Calves C 44 and C 45 became blind on a ration free from cottonseed meal. C 44 became completely blind at 347 days of age and C 45 at 293 days of age. Neither of these calves died from the effects of the ration but their condition was so poor that C 45 was killed and the ration of C 44 changed.

The histopathological study of the glands and organs of calves C 43 and C 45 and C 47 made by Dr. Delez of the Department of Animal Pathology (37) seemed to indicate that the optic nerves, kidneys, and liver are the most seriously and frequently affected organs. Sections of the wall of the rumen, intestines, adrenal, thyroid, pineal, and the thymus failed to show more than very slight, if any, abnormal changes. The testes of C 43 and C 45 were apparently normal, while those of C 47 showed considerable cellular infiltration of the intertubular tissue.

The liver changes consisted of: Considerable congestion which extended some distance below the capsule into the parenchyma. In the region underneath the capsule, a number of pyknotic cells were seen. There was considerable degenerative change of the parenchyma cells. There was a cellular infiltration of the portal sheaths underneath the capsule, as well as a fibriotic condition around some of the bile ducts.

A section of one of the optic nerves of C 43 showed a degeneration (plate 1) of the myelin sheaths in the center of the nerve.



This degenerative change probably did not extend throughout the entire nerve, since a section from another portion of the same nerve did not reveal this change. A parallel section of the right optic nerve of C 45 (plate 2) showed a constricted portion. Since C 47 was not blind, no portion of the optic nerve was saved for histological study.

Pathological changes in the kidneys are shown in plates 3, 4, 5, and 6. Foci of intertubular proliferation (focal interstitial

TABLE 2  
*Feed record of calves fed corn gluten meal and corn distillers' grain with wheat straw a roughage*

Lot 2—Part I

30-DAY PERIODS	C 44						C 45					
	Skim milk	Corn gluten	Corn distillers	Yellow corn	Oats	Wheat straw	Skim milk	Corn gluten	Corn distillers	Yellow corn	Oats	Wheat straw
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1	360	17	35	19	19	Ad. lib.	360	6	33	23	23	Ad. lib.
2	360	60	15	30	30	Ad. lib.	360	51	21	27	27	Ad. lib.
3	360	70	5	35	35	Ad. lib.	360	61	10	36	36	Ad. lib.
4		93	0	45	45	Ad. lib.	120	90	0	38	38	Ad. lib.
5		84	13	42	42	66		69	5	38	38	Ad. lib.
6		68	17	34	34	72		68	17	34	34	66
7		77	19	38	38	84		75	18	37	37	78
8		80	20	40	40	90		35	9	17	17	36*
9		80	20	40	40	90						
10		77	19	38	38	87						
11		75	18	37	37	84						
12		80	20	40	40	90						

\* Twenty days.

nephritis) were present. Atrophy of the tubular epithelium of the proximal tubules was noted. Those were dilated and contained albumin (chronic parenchymatous nephritis). A few areas along the surface of the cortex showed cellular infiltration (mononuclears and a few lymphocytes, but no polymorphs). The glomerular capsules in the area involved were thickened and there was also a thickening of the renal capsule covering this area. The evidence seems to indicate a chronic interstitial nephritis.

## Part II

The results of Part I indicated that the cause of cottonseed meal injury in cattle may be due to too heavy feeding of concentrates in proportion to roughage. In order to determine the effects of feeding cottonseed meal in reasonable amounts with ample roughage of high quality, ten grade Holstein calves were placed on experiment at about 90 days of age. The inheritance of the animals in both lots was similar. Eight of the heifers were sired by the same bull. The dams of all the animals used in this experiment were related.

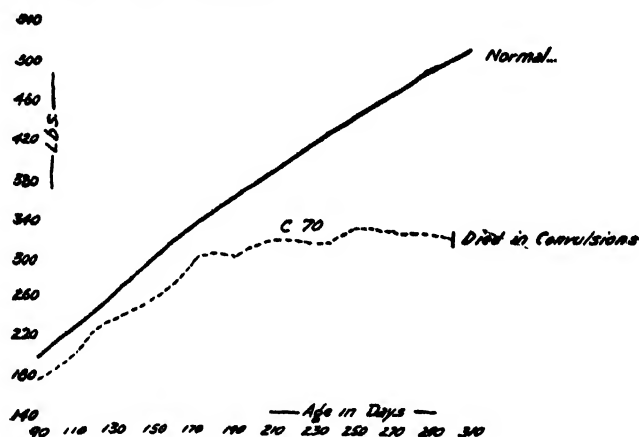


FIG. 3. SHOWING EFFECT OF THE COTTONSEED MEAL USED IN THIS INVESTIGATION ON GROWTH AND HEALTH WHEN FED WITH A ROUGHAGE OF POOR QUALITY

These animals were divided into two lots of five heifers each. Animals in lot 1, G 1, G 3, G 5, G 7, and G 9, received cottonseed meal as the principal source of protein. Those in lot 2, G 2, G 4, G 6, G 8, and G 10, received linseed oil meal as the principal source of protein. The latter lot was used as a check since linseed oil meal is recognized as a safe protein concentrate to use as a supplement for dairy calves.

**Rations.** Both lots received skim milk until about 150 days of age. They were also fed throughout the experiment all the timothy hay and corn silage which they would clean up. Bone

meal was fed as 2 per cent of the protein concentrate to both lots of heifers. They were allowed free access to salt. Yellow corn was fed in order to equalize total digestible nutrients. The animals in lot 1 received 0.5 pound of cottonseed meal per day until 150 days of age, after which enough cottonseed meal was fed

TABLE 3

*Feed record of calf C-70, which was fed principally from the shipment of cottonseed meal used in this investigation*

10-DAY PERIODS	SKIM MILK	COTTONSEED MEAL†	CORN	OATS	WHEAT STRAW
	pounds	pounds	pounds	pounds	pounds
1*	120	4.6	10	10	3
2	120	11.5	10	10	5
3	120	12.0	10	10	11
4	120	25.0	10	10	9.5
5	120	25.0	9	9	7
6	88	28.0	10	10	11.5
7		35.0	10	10	17.5
8		42.5	10	10	25
9		45.0	10	10	18
10		40.5	9	9	16
11		40.5	9	9	16
12		45.0	10	10	12
13		45.0	7	7	12
14		45.0	9	9	10
15		45.0	10	10	10
16		45.0	10	10	10
17		40.5	9	9	9
18		27.0	6	6	7
19		27.0	6	6	7
20‡		9.0	2	2	4

\* Age at beginning of experiment, 96 days.

† Two per cent steamed bone meal and 1 per cent salt were added to the cottonseed meal.

‡ Four days.

to meet the protein requirement according to the Armsby feeding standard. The protein furnished in the roughage and corn was allowed as excess protein. The cottonseed meal used in this experiment during the first nine months of the experiment was from the same lot as that fed C 43 and C 47. The shipment used later was tested biologically by feeding it to calf C 70.

C 70, a Holstein bull calf, was placed on a ration of cottonseed meal, wheat straw, corn, oats, bone meal, and salt at 96 days of age. Skim milk was also fed until 150 days of age. Figure 3 gives the growth of this animal. Food consumption for C 70 is reported in table 3. Death occurred as a result of convulsions at 301 days of age.

Old process linseed oil meal was fed to the animals in lot 2 as the chief source of protein supplement. These heifers were fed

TABLE 4

*Growth and feed records of animals fed cottonseed meal or linseed oil meal along with a ration otherwise adequate*

NUMBER OF ANIMAL	GROWTH RECORD						FEED RECORD TO MONTHS OF AGE							
	At the begin- ning	Initial weight	Per cent nor- mal*	Weight at 18 months	Per cent nor- mal*	Average daily gain	Skim milk	Cottonseed meal	Linseed oil meal	Corn	Silage	Timothy	Salt	
	days	lbs.		lbs.		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	
G 1	118	245	99.71	811	118.23	1.34	396	918.3			178	4,373	3,091	16
G 3	90	169	84.5	807	117.64	1.42	723	923.7			242	4,474	3,102	9
G 5	89	146	73.54	732	106.71	1.09	742	909.5			309	3,543	2,919	11
G 7	89	161	81.09	701	102.19	1.00	990	872.0			299	3,516	2,723	23
G 9	94	190	91.99	747	108.9	1.25	960	863.7			288	3,370	2,784	20
G 2	119	300	121.3	922	134.41	1.48	384		1,176.2	19	4,414	3,097	14	
G 4	93	203	99.08	831	121.14	1.40	741		1,162.7	168	4,346	3,009	12	
G 6	94	198	95.87	779	113.55	1.30	720		1,113.9	136	3,800	3,013	11	
G 8	89	165	83.11	763	111.23	1.32	978		1,090.7	188	3,616	2,860	23	
G 10	92	199	97.91	832	121.29	1.44	720		1,108.3	140	3,864	2,989	18	

\* Eckles, C. H. 1920. University Missouri Agr. Exp. Sta. Research Bul. 36.

0.7 pound daily until 150 days of age, after which the intake was gradually raised to three pounds of linseed oil meal per day.

*Health.* There has been but very little difference in the health of the two lots of heifers thus far. The animals which have received 2.4 pounds of cottonseed meal daily for some time have failed to show swollen joints, stiffness, blindness, or convulsions, which are the symptoms of so called cottonseed meal injury in cattle. Growth in weight often reflects the health of the animal. The gain in body weight of the heifers in lots 1 and 2 is shown in



TABLE 5—Continued

Lot 2

G 2—AGE 256 DAYS			G 4—AGE 226 DAYS			G 6—AGE 117 DAYS		
2.9 pounds linseed oil meal daily			2.7 pounds linseed oil meal daily			0.6 pound linseed oil meal daily		
Time after feeding dye		Color	Time after feeding dye		Color	Time after feeding dye		Color
hours	min-utes		hours	min-utes		hours	min-utes	
7	30	0	2	30	0	2	30	0
10	15	0	7	30	0	7	30	0
11	45	0	10	30	0	9	0	0
15	0	++	13	45	++++	10	30	0
17	30	++++	17	25	+++++	12	0	0
23	0	+++++	19	0	++	14	5	+
25	0	+++++	22	30	+++++	15	0	++
28	30	+++++	23	0	+++++	16	30	++
33	0	+++++	25	15	+++++	18	40	++++
41	0	+++++	30	0	+++++	23	0	+++++
47	0	++	33	30	++++	25	15	+++++
50	30	+	36	0	+++	27	0	+++++
56	0	0	40	10	+++	34	10	+++++
57	45	0	42	0	++	37	0	+++++
58	30	0	46	30	+	37	10	+++++
62	30	0	47	0	+	40	15	+++++
			54	25	0	42	45	+++++
			56	30	0	48	0	+++++
			60	10	0	56	55	++
						60	15	+

++++ abundant dye; +++ large amount; ++ relatively large; + small amount; 0 absent.

fed. Ten recognized judges of cattle attempted to differentiate between the heifers fed linseed and those fed cottonseed meal. The difference between the sleekness of coat, pliability of hide, and condition of the animals was so small that the judges were unable to distinguish between the two lots of heifers. The double thickness of hide was measured from time to time by a pair of calipers. The measurement was taken at the mid-section of the last rib. No difference in double thickness was observed between the animals fed linseed and those fed cottonseed meal.

Lice infestation in cattle depends to some extent on the health of the skin. When the sebaceous glands secrete sufficient oil to

keep the hair oily, lice can not exist. The oil clogs up the breathing mechanism of the lice, therefore, any feeding regime which keeps down lice infestation probably does so by increasing the secretory activity of the sebaceous glands of the skin. In this experiment, there was no appreciable difference in lice infestation between the linseed and the cottonseed meal fed heifers.

*The rate of food passage through the digestive track.* It is common opinion among investigators that linseed oil meal has a laxative effect and cottonseed meal a costive effect when fed to cattle. Therefore, in this work the rate of food passage through the digestive tract and the consistency of feces of the two lots of heifers were studied.

Several methods have been suggested and tried for determining the rate of food passage in ruminants. None of these has proved satisfactory.

Ewing and Smith (38) attempted to use small rubber washers as markers. Some of the rubber washers were passed in from twelve to sixty hours, and some were not passed at all. These investigators later adopted the slaughter method. Fish (39) fed Sudan III to animals prepared for slaughter and noted the presence of the dye in various portions of the digestive tract.

A modification of the method suggested by Fish was used in this work. One gram of the fat soluble dye Sudan III was shaken with 100 cc. of ethyl ether. This amount of ether did not dissolve all the dye. However, the dye was held in suspension for a short time. This dye suspension was quickly and thoroughly mixed with a feed of either linseed or cottonseed meal. The ether was permitted to evaporate from the feed. The feed containing Sudan III was fed in the morning immediately following the feeding of silage.

Samples of feces were then collected from each passage and the time noted. These samples were dried in an oven at 80 to 90°C. One gram of the dried feces was then extracted with 10 cc. of ethyl ether. The residue was filtered off. The color of this extract from the first sample of feces was green, due to the chlorophyll from the hay. The red color of Sudan III superimposed upon the green produced a light brown to a brownish red color.

The color of the ether extracts of the feces samples was compared in order to determine the rate of food passage of the two rations. Only three animals in each group were used in the first trial. The results are reported in table 5.

The average initial appearance of the dye in the feces was fourteen hours, twenty minutes in both groups. The average final appearance of the dye was fifty-two hours, thirty-five minutes in the group receiving linseed oil meal and forty-eight hours, twenty minutes in the group receiving cottonseed meal. A similar trial was conducted on all ten heifers eight months later when the animals in lot 1 were receiving 2.4 pounds of cottonseed meal per head and those in lot 2 were receiving 3 pounds in linseed oil meal per head. The initial appearance of the dye in this trial was approximately fifteen hours, thirty minutes and the final appearance was fifty-five to sixty hours in both groups.

Thus far in this work there has been very little difference in the consistency of feces excreted by animals receiving cottonseed meal and linseed oil meal. The feces of all the animals have been normal throughout the experiment.

#### DISCUSSION

There appears to be a close relationship between the symptoms of cottonseed meal injury in cattle and the injury produced when too much concentrates are fed in proportion to roughage. Convulsions, stiffness, and blindness which are the common symptoms of cottonseed meal injury in cattle are also manifested when the ration contains a considerable amount of concentrates and little or no hay or grass. Blindness and stiffness of gait were manifested by the two calves C 44 and C 45 even though no cottonseed meal was fed. These findings are in harmony with those of the North Carolina Station (36) where similar symptoms were produced in cattle fed either soybean meal or peanut meal with a roughage of poor quality. The cause of the deficiency in cattle fed such rations is not definitely known.

Davenport (40) concluded from his investigations that bulk was necessary. McCandlish (41) observed that calves could not be grown from birth to maturity on milk alone. The addition of



certain grains only intensified the deficiency. Vitamin supplements were of little value. Eckles (42) reported favorable results when calcium carbonate was added to the ration of a calf. However, the addition of vitamins had no apparent beneficial effect. Huffman and Robinson (43) were unable to raise calves on concentrates alone or with concentrates supplemented with calcium carbonate, bone meal, or cod liver oil. The addition of bone meal to the ration of C 70 failed to prevent the symptoms of cottonseed meal poisoning.

Our results suggest that cottonseed meal may be fed to growing calves without injury. The five heifers receiving 2.4 pounds of cottonseed meal throughout most of their growing period along with an otherwise adequate ration failed to show the symptoms of cottonseed meal injury. Although the gain made by this group was not as great as that made by the animals receiving linseed oil meal, the difference was negligible in view of the fact that the smaller and slightly less thrifty calves received cottonseed meal in the ration. Since the animals in this group are all above normal in weight, the difference in the two groups can easily be explained on the basis of individual variations. The purpose of this work was not to bring out the relative efficiency of the proteins of cottonseed meal and linseed meal.

The results of this experiment indicate that at least two pounds of cottonseed meal per day can be fed without injury to calves which have reached five months of age, when plenty of good hay is fed along with silage. Apparently, roughage in sufficient quantity and of the proper quality neutralizes the bad effects of concentrates.

These results are in harmony with the results obtained by the North Carolina Experiment Station (36) where such supplements as pasture, hay, minerals, and vitamins made possible the heavy feeding of cottonseed meal without injurious results. Gossypol, which many investigators have found to be the poisonous principle in the seed of cotton, is probably not the cause of cottonseed meal injury brought about by the heavy feeding of cottonseed meal to cattle. So-called cottonseed meal injury in cattle is probably due to dietary deficiencies in the ration. The results

of this experiment indicate that such deficiencies in cattle may be overcome by feeding a sufficient amount of corn silage and hay of good quality.

The results of this investigation are not in harmony with the generally accepted idea that the feeding of linseed oil meal makes a sleeker coat and a more pliable hide than when cottonseed meal is fed. There was very little difference between the two lots in this respect. These results agree with the findings of Curtiss (45) who concluded that cottonseed meal had the same effect when fed to horses as linseed oil meal, making the coat smooth and glossy.

The results also failed to demonstrate the idea that linseed oil meal has a more laxative effect than cottonseed meal. There was no appreciable difference between the two groups in the rate of food passage, either in the initial appearance of a feed in the feces or in the lag of feed in the digestive tract. About fifteen hours were required in both groups of animals for the initial appearance of the dye used as an indicator and the lag was from fifty to sixty hours in all cases. The consistency of the feces in the two groups was practically the same even when the lot which was fed cottonseed meal received 2.4 pounds of cottonseed meal daily and the other group received three pounds of linseed meal daily. However, animal C 43 had diarrhea from time to time on a ration consisting of 6 pounds of cottonseed meal daily along with corn, oats, and wheat straw. Other investigators have observed this condition among cattle fed heavily on cottonseed meal. (32) (44) Curtis (45) reported that cottonseed meal was laxative when fed to horses.

#### SUMMARY

Cottonseed meal injury in cattle is similar, if not identical, to the injury produced when too much concentrates in proportion to roughage is fed.

At least two pounds of cottonseed meal daily can be fed to calves five months of age or older which receive all the silage and hay of good quality they will eat.

There was no appreciable difference in the sleekness of coat

and pliability of hide between the heifers receiving cottonseed meal and linseed meal.

There was no appreciable difference between the rate of food passage through the digestive tract by the heifers receiving cottonseed meal and linseed oil meal.

There was no measurable difference in the consistency of feces excreted by the two groups of heifers.

#### REFERENCES

- (1) Unpublished data: Dairy Dept., Mich. Agr. Exp. Sta.
- (2) NEVENS, W. B.: Jour. Dairy Sci., iv, 375 (1921).
- (3) ABDEKHALDEN, E., AND ROSTOSKI, O.: Zeit. Physiol. Chem., xlv, 265 (1905).
- (4) RICHARDSON, A. E., AND GREEN, H. S.: Jour. Biol. Chem., xxv, 307 (1916).
- (5) MENDEL, L. B.: Jour. Am. Med. Assoc., lxiv, 1539 (1915).
- (6) OSBORNE, T. B., AND MENDEL, L. B.: Jour. Biol. Chem., xxix, 69 (1917).
- (7) MCCOLLUM, E. V., AND SIMMONDS, N.: Jour. Biol. Chem., xxxii, 347 (1919).
- (8) DINWIDDIE, R. R., AND SHORT, A. K.: Ark. Exp. Sta. Bul. No. 108 (1911).
- (9) JONES, J. M., LUSH, J. L., AND JONES, J. H.: Texas Agr. Exp. Sta. Bul. No. 309 (1923).
- (10) COMBS, S., AND CURTISS, R. S.: Jour. Dairy Sci., iv, 334 (1921).
- (11) McNUTT, J. C.: North Carolina Exp. Sta. Rpt. No. 34, 33 (1911).
- (12) KENNEDY, W. J., AND MARSHALL, F. R.: Iowa Agr. Exp. Sta. Bul. No. 66, 234 (1902).
- (13) CRAWFORD, A. C.: Jour. Pharmacol. and Exp. Ther., i, 547 (1910).
- (14) WITHERS, W. A., AND RAY, B. J.: Jour. Biol. Chem., xiv, 53 (1913).
- (15) FORBES, E. B., BEEGLE, F. M., AND MENSCHING, J. E.: Ohio Agr. Exp. Sta. Bul. 255, 227 (1913).
- (16) WELLS, C. A., AND EWING, P. V.: Ga. Exp. Sta. Bul. No. 119 (1916).
- (17) ROMMEL, G. M., AND VEDDER, E. B.: Jour. Agr. Res., v, 489 (1915).
- (18) BECHDEL, S. I., ECKLES, C. H., AND PALMER, L. S.: Jour. Dairy Sci., ix, 409 (1926).
- (19) MCGOWAN, J. P., AND CRICHTON, A.: Biochem. Jour., xviii, 272 (1924).
- (20) WITHERS, W. A., AND CARRUTH, F. E.: Jour. Biol. Chem., xxxii, 245 (1919).
- (21) WITHERS, W. A., AND BREWSTER, J. F.: Jour. Biol. Chem., xv, 161 (1913).
- (22) WITHERS, W. A., AND CARRUTH, F. E.: Jour. Agr. Res., v, 261 (1915).
- (23) WITHERS, W. A., AND CARRUTH, F. E.: Jour. Agr. Res., xii, 83 (1918).
- (24) SCHWARTZ, E. W., AND ALSBERG, C. L.: Jour. Agr. Res., xxviii, 173 (1924).
- (25) SHEERWOOD, F. W.: Jour. Agr. Res., xxxii, 793 (1926).
- (26) MENAUL, P.: Jour. Agr. Res., xxvi, 233 (1923).
- (27) DOWELL, C. T., AND MENAUL, P.: Jour. Agr. Res., xxvi, 9 (1923).
- (28) GALLUP, W. D.: Jour. Dairy Sci., ix, 359 (1926).
- (29) EDGERTON, C. W., AND MORRIS, H.: La. Sta. Bul. No. 134 (1912).
- (30) OSBORNE, T. B., AND MENDEL, L. B.: Jour. Biol. Chem., xxix, 289 (1917).
- (31) NEVENS, W. B.: Jour. Dairy Sci., iv, 552 (1921).
- (32) WRIGHT: Ark. Exp. Sta. Bul. No. 9 (1889).

- (33) STANLEY, E. B.: Ariz. Agr. Exp. Sta. Bul. No. 93 (1921).
- (34) BURTIS, F. C.: Okla. Exp. Sta. Bul. 58, 38 (1903).
- (35) MICHELS, J.: North Carolina Agr. Exp. Sta. Rpt. No. 33, 29 (1910).
- (36) North Carolina Exp. Sta. Rpt. No. 43, 22 (1925).
- (37) DELEZ, A.: Thesis for M. S. Degree, Mich. State College (1928).
- (38) EWING, P. V., AND SMITH, F. H.: Jour. Agr. Res., x, 55 (1917).
- (39) FISH, P. A.: Cornell Veterinarian, xiii, 83 (1923).
- (40) DAVENPORT, E. V.: Ill. Agr. Exp. Sta. Bul. No. 46 (1897).
- (41) McCANDLISH, A. C.: Jour. Dairy Sci., vi, 54 (1923).
- (42) ECKLES, C. H.: Quoted by HENRY, W. A., AND MORRISON, F. B.: Feeds and Feeding, 18th edition, 432 (1923).
- (43) HUFFMAN, C. F., AND ROBINSON, C. S.: Jour. Biol. Chem., lxi, 101 (1926).
- (44) GRAY, D. F., AND WARD, W. F.: Ala. Exp. Sta. Bul. No. 158 (1911).
- (45) CURTISS, R. S.: North Carolina Exp. Sta. Bul. No. 216 (1911).

PLATE 1

CROSS-SECTION OF OPTIC NERVE OF ANIMAL C 43 ENLARGED 125 TIMES, SHOWING  
DEGENERATION OF MYELIN SHEATHS IN CENTER OF OPTIC NERVE

This animal received a ration of cottonseed meal, corn, oats, and wheat straw.  
G 1, G 3, G 5, G 7, and G 9 received cottonseed meal for nine months after the  
experiment started from the same shipment fed C 43.

PLATE 2

SHOWING CONSTRICTION OF OPTIC NERVE IN ANIMAL C 45, ENLARGED 30 TIMES

This animal received a ration of corn gluten, corn distillers grain, corn, oats,  
and wheat straw.

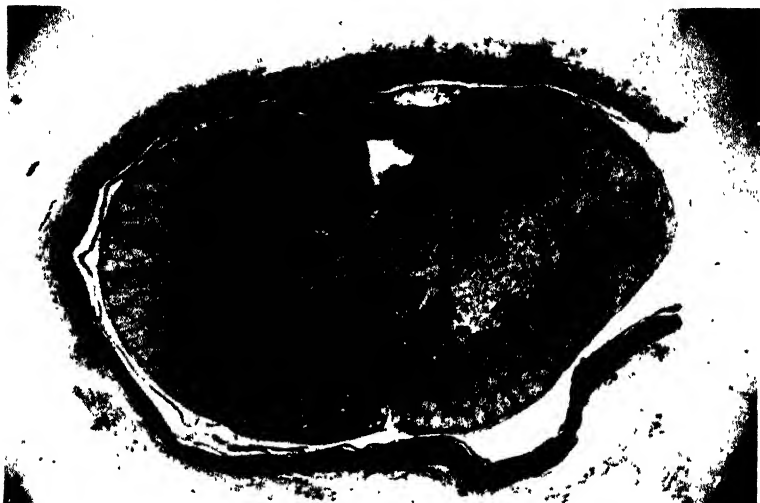


PLATE 1

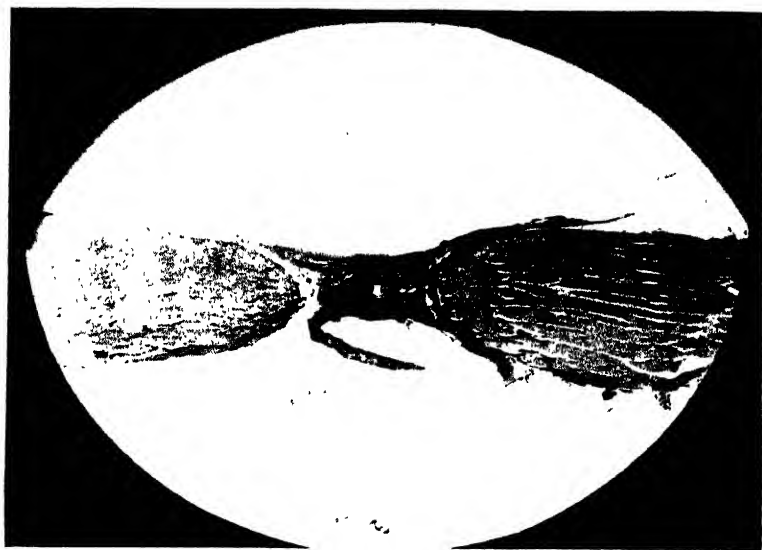


PLATE 2

PLATE 3

C 43. SECTION OF KIDNEY, ENLARGED 800 DIAMETERS

*A*, intertubular proliferation; *B*, thickened and distorted Bowman capsule; *C*, parenchymatous degeneration of tubular epithelium

PLATE 4

C 45. SECTION OF KIDNEY, ENLARGED 800 DIAMETERS

*A*, focus of cellular infiltration; *B*, thickened Bowman's capsule; *C*, parenchymatous degeneration of tubular epithelium.



PLATE 3

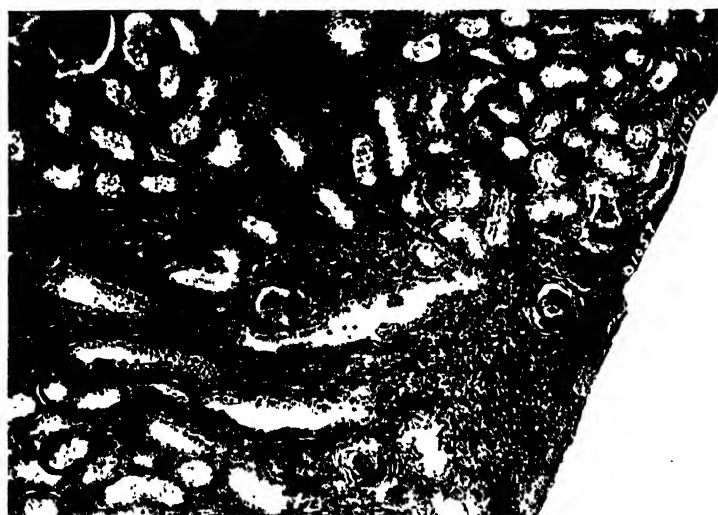


PLATE 4



PLATE 5

C 43. SECTION OF KIDNEY, ENLARGED 1000 DIAMETERS

Focus of cellular infiltration

PLATE 6

C 45. SECTION OF KIDNEY, ENLARGED 750 DIAMETERS

Focus of cellular infiltration

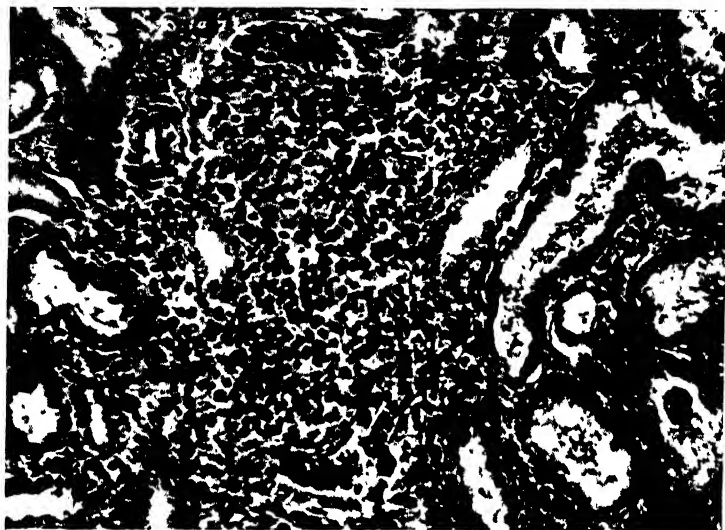


PLATE 5



PLATE 6

PLATE 7

G 3 AT BREEDING AGE (FIFTEEN MONTHS)

Principal source of protein: Cottonseed meal

PLATE 8

G 4 AT BREEDING AGE (FIFTEEN MONTHS)

Principal source of protein: Linseed meal



PLATE 7

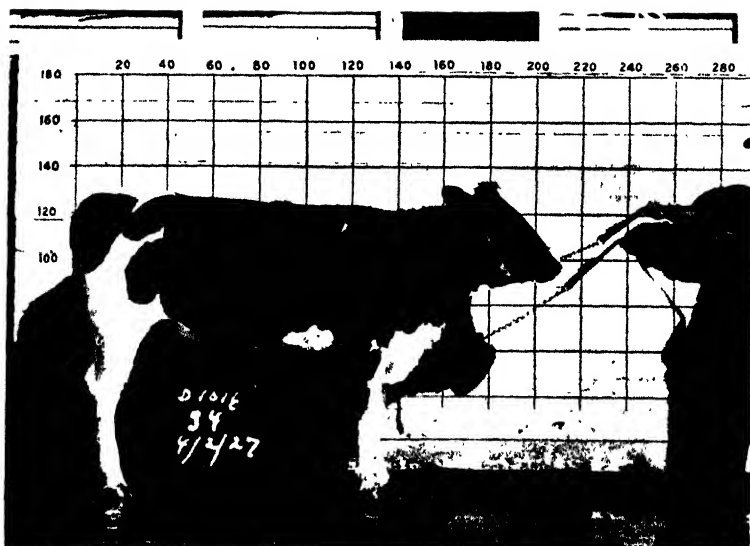


PLATE 8

# SOME OBSERVATIONS ON THE BACTERIAL CONTENT OF DRIED MILK\*

H. MACY

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The removal of the water from milk during the process of drying leads to transportation economies, and enhanced keeping qualities. The commercial life of fluid milk is decidedly limited, particularly because many microorganisms are capable of rapid alteration of a food stuff with such a high water content. The development of microorganisms in the dehydrated product is precluded since the percentage of moisture in the powdered milk is far below the requirements of the organisms commonly present in milk. Consequently, it is to be expected that deterioration of dried milk must be explained on bases other than microbiological.

However, the bacterial content of the dried milk should be taken into account, especially the survival of microorganisms during long periods of storage and the possibility of subsequent fermentation caused by them when the product is reconstituted by the addition of water up to the normal concentration of moisture of fluid milk.

With this point in mind a number of samples of dried milk purchased in local stores or obtained directly from the manufacturers or distributors have been studied to determine the bacterial content, before and after periods of storage.

## REVIEW OF LITERATURE

A considerable volume of literature has accumulated upon the manufacture, composition and utilization of dried milk. Data upon the bacterial content of this product, however, are not very extensive.

Delepine (3) has reported upon some careful studies of the effect of different methods of manufacture upon the bacterial content of

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milk during the drying process, with particular emphasis placed upon the destruction of the tubercle bacillus. He has shown that a whole milk dried by the spray process yielded a product which contained from 10,000 to 15,200 bacteria per gram when taken directly from the drying chamber. Downs (4) found that the bacterial count of spray process powder, prepared from milk previously pasteurized and condensed, ranged from 15,600 to 595,000 per gram, with an average of 178,000. Milk dried by the same process, but not condensed before subjection to the spraying process yielded a product containing 1,500,000 to 3,500,000 bacteria per gram or an average of 2,269,000. Tillmans and Strohecker (12) report counts of 5000 to 30,000 per gram for powder prepared by the Krause process, where the milk was delivered into a drying chamber from rapidly rotating discs.

An anonymous report (1) from England on milk dried by the Hatmaker or hot drum process indicates that the product so obtained gave a count of 70 to 120 per cubic centimeter when reconstituted to the normal moisture content of fluid milk. Delepine (3) found that dried whole milk directly from the rollers (Just-Hatmaker process), contained 70 to 300 bacteria per gram but after it had been powdered the count had increased to 5950 to 14,600. Dried skim milk prepared in the same way gave a count of 10 to 100 per gram when taken from the rollers. Downs (4) studied powder made by the modified Kunick process and found 16,900 to 626,000 bacteria per gram with an average of 49,500. The experiments of Grosso (5) upon Just-Hatmaker dried milk revealed 4000 to 5400 per gram. Hoffman (6) made counts upon the product made by the same process and found 4000 to 5000 per gram in dried whole milk and 2200 to 6000 per gram in dried skim milk. One sample of Just-Hatmaker powder studied by Hueppe (7) gave maximum counts of 4100 per gram on gelatin plates, and 6400 on agar. The minimum counts were 800 to 1000 per gram. Jephcott and his co-workers (8) report the counts obtained from reconstituted milk made from dried milk manufactured by the drum process during three autumn months. The counts in September were 0 to 565 per cubic centimeter, in October, 0 to 275 and in November, 0 to 198, with an average for the three months of 25 per cubic centimeter. Kossowicz (9)

found that Just-Hatmaker dried milk contained 45 to 80 bacteria per gram when the product was taken directly from the drums and 750 to 1,250 per gram when taken from the collecting boxes. Supplee and Ashbaugh (11) made analyses upon a considerable number of samples prepared by the Just-Hatmaker process. The counts obtained from 158 samples, within one day after manufacture varied from 350 to 170,000 per gram, averaging 10,100. Ten samples taken directly from the cylinders averaged 561 per gram. Seven samples were also studied to show progressive contamination in handling. The dried milk taken directly from the drums averaged 563 per gram, after sifting 1614 and after packing 3271 bacteria per gram.

A report by Coutts (2) gives the counts obtained from a number of samples of dried milk but the process by which they were made is not mentioned. Full cream dried milk reconstituted (1:9) gave counts of 0 to 11,900 per cubic centimeter for aerobes at 37°C. for forty-eight hours incubation; 100 to 86,400 per cubic centimeter at 22°C. for ninety-six hours; for anaerobes 0 to 300 per cubic centimeter at 37°C. for forty-eight hours; and 0 to 890 per cubic centimeter at 22°C. for ninety-six hours. Five samples of the completely or partially skimmed product when reconstituted (1:7) showed 0 to 892,000 bacteria per cubic centimeter for aerobes at 37°C. for forty-eight hours incubation; and 100 to 757,200 per cubic centimeter at 22°C. for ninety-six hours; for anaerobes 0 to 38,800 per cubic centimeter at 37°C. for forty-eight hours and 0 to 59,960 per cubic centimeter at 22°C. for ninety-six hours. Likewise, Prachfeld (10) reports for commercial powder, without stating the process by which it was made, 4071 to 21,700 bacteria per gram of powder.

Delepine (3) observed the changes taking place in the bacterial count of drum-dried whole milk during a storage period of 112 days. The counts are based upon the number of bacteria per cubic centimeter of reconstituted milk and are as follows:

	COUNT PER CUBIC CENTIMETER	
	Gelatin plates 20°C.	Agar plates 37°C.
Fresh.....	4,900	800
112 days.....	4,180	500-600

Kossowicz (9) found that dried milk which contained 750 to 1,250 bacteria per gram before storage, showed after two months a count of 450 to 930 per gram, while that preserved in soldered tin boxes had a count of 1,850 to 2,460 per gram. Supplee and Ashbaugh (11) studying 43 samples of Just-Hatmaker dried milk during a period of a year obtained the following average counts per gram of powder:

FRESH	2 MONTHS	4 MONTHS	6 MONTHS	8 MONTHS	10 MONTHS	12 MONTHS
22,508	7,818	2,487	655	412	354	261

They point out that there is some difference in the rapidity of the decrease in count depending upon the moisture content of the sample.

#### EXPERIMENTAL DATA

##### *Sources of samples*

For the studies which are reported herewith, samples of dried milk were obtained from a variety of sources. Among them were several samples from Canada, Holland, Italy and New Zealand. The majority of the samples were purchased locally, some were presented by the manufacturers, while others had been on the shelves of the laboratory for several years. Few of the samples gave any clue as to the date of manufacture, consequently nothing was known about their past history. They were simply representative samples that might be found on the shelves of any market.

##### *Methods of analysis*

The containers were thoroughly wiped with a 5 per cent phenol solution, wiped dry and flamed carefully before they were opened. Wherever a can opener was required, it was sterilized before use. After the containers were opened, they were protected from contamination while being mixed thoroughly with a sterile wooden spatula. A 10-gram sample was weighed on sterile oiled weighing paper then placed in a 250 cc. wide-mouth, glass stoppered bottle. Ninety cubic centimeters of sterile water warmed to 35 to 40°C.



were added slowly. The dried milk was dissolved as thoroughly as possible and platings made with the necessary dilutions. Clay-top plates were used in order to reduce spreaders to a minimum. The plates were poured with milk powder agar and

TABLE 1  
*Bacterial content of domestic dried milk (spray process)*  
Number of bacterial colonies per gram of dried milk

ORIGINAL	AFTER STORAGE AT ROOM TEMPERATURE FOR						
	6 months	1 year	2 years	3 years	4 years	5 years	6 years
5,500,000 (S)	2,400,000		74,000				1,700
3,100,000 (W)		2,200				1,310	
2,800,000 (W)		36,500	5,000	2,300			
500,000 (W)		226,000					290
490,000 (S)			625,000	164,000	38,000		
400,000 (W)		200				480	
250,000 (S)		400		950		280	
157,000 (S)		162,000	11,100	18,500			
84,000 (W)			4,500	2,800			
54,000 (W)		3,800		2,200			
52,000 (W)			1,590		8,000		
51,000 (W)	3,500	2,800			2,250		
50,000 (W)			650	625	180		
42,000 (W)	19,500		300		100		20
34,000 (W)	15,200	1,500		2,000			
29,000 (W)		3,700	3,000	620			
27,600 (W)	3,100	2,000					
27,600 (W)	5,000			1,850	495		
18,400 (W)	3,000	2,300				170	
8,200 (W)		16,000	60	200			
8,000 (W)	2,700		100		85		10
7,100 (S)		770	165				
6,200 (S)		750	220	180			
4,400 (W)							

W = whole milk; S = skimmed milk.

incubated in 20°C. for five days and for an additional two days at 37°C. after which they were counted. The results are reported in terms of the number of bacterial colonies per gram of powder.

The counts were not always easy to make because of the persistence of spreaders, even when the clay-top plates were used. The dehydration of the agar in the plates was checked as far as

possible by placing open dishes of water on top of each pile of plates. Even with this precaution some plates dried badly. The counts that are reported were made only from satisfactory plates. The results of many analyses had to be discarded because of the drying or the spreaders.

TABLE 2  
*Bacterial content of foreign dried milk (spray process)*  
Number of bacterial colonies per gram of dried milk

ORIGINAL	AFTER STORAGE AT ROOM TEMPERATURE FOR			
	1 year	2 years	3 years	4 years
3,100,000 (S)		80,000	350	300
750,000 (W)		20,000	400	120
660,000 (W)		7,700	9,750	
79,000 (W)		1,600	7,000	
61,000 (W)	63,000	6,300	140	
11,000 (S)		1,200	11,800	
5,400 (S)	5,000	2,600	2,100	

W = whole milk; S = skimmed milk.

TABLE 3  
*Bacterial content of domestic dried milk (drum process)*  
Number of bacterial colonies per gram of dried milk

ORIGINAL	AFTER STORAGE AT ROOM TEMPERATURE FOR				
	6 months	1 year	2 years	3 years	4 years
2,200 (W)	200	100		80	
2,050 (W)	1,470		200		85
320 (W)			80	60	30
240 (W)			100	90	28
40 (W)		40			

W = whole milk; S = skimmed milk.

*Bacterial counts of powders made by the spray process*

The original counts on the spray powders show a wide range as indicated in column one of tables 1 and 2. Both the domestic and foreign samples show this tendency. On the plates prepared from the high count samples there was a noticeable predominance of small subsurface colonies, resembling those of *S. lactis*. Sterile

milk inoculated with these colonies, in the majority of cases, was coagulated and acid in reaction. In many of the plates, the flora resembled closely the flora of freshly drawn milk. Samples known to be old had a more restricted flora. It was interesting to find one sample in a sealed can, known to be at least twelve years old, which gave a count of 52,000. The data indicate that the bacterial content of spray powder may be expected to be in

TABLE 4  
*Bacterial content of foreign dried milk (drum process)*  
Number of bacterial colonies per gram of dried milk

ORIGINAL	AFTER STORAGE AT ROOM TEMPERATURE FOR			
	1 year	2 years	3 years	4 years
7,900 (W)	140		20	40
7,800 (W)	60		10	70
420 (W)	230	110		60
350 (W)		50	80	10
310 (W)	410	180		50
250 (S)		40	50	25
250 (W)				
240 (W)	170	90		

W = whole milk; S = skimmed milk.

the thousands per gram and sometimes in the millions. This is in accordance with the reports of others. A diminution in the number of bacteria appears to take place more rapidly after the sealed package has been opened one or more times.

*Bacterial counts of dried milk made by the drum process*

Only a few samples of the drum process product were studied. The results are given in tables 3 and 4. The domestic and foreign samples were quite similar. Most of the original counts were in the hundreds with an occasional sample showing counts of a few thousands. This likewise, corresponds with other data in the literature.

*Bacterial counts of dried milk after storage*

Many of the samples were retained for a period of time depending upon the quantity of the sample. They were left in a pro-

tected place at room temperature during this time. The samples were kept in the original containers or placed in glass stoppered bottles. Analyses were made at intervals over a period of six years. It was impossible to make a count on every sample each year because the quantity of the samples was limited. Irregular periods of storage for the various samples were decided upon to give a wider range of data.

The results of these analyses are shown in tables 1 to 4. It will be noted that there is a decided decrease in most spray process

TABLE 5  
*Reduction in counts after various periods of storage*

TYPE OF DRIED MILK	AFTER STORAGE AT ROOM TEMPERATURE FOR						
	6 months	1 year	2 years	3 years	4 years	5 years	6 years
	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Spray.....	56.3	90.7	93.9	97.4	99.2	99.9	99.9
Drum.....	60.0	96.0	97.6	97.9	97.9		

TABLE 6  
*Types of bacteria present in dried milk*

TYPE OF DRIED MILK	NEUTRAL	NEUTRAL PEPTON- IZING	WEAK ACID	ACID COAGU- LATING	ACID PEPTON- IZING	ALKA- LINE	ALKA- LINE PEPTON- IZING
	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Spray.....	11.0	2.4	26.0	26.8	12.8	7.3	13.7
Drum.....	22.5	6.2	15.1	5.2	17.4	8.9	24.7

samples during the first year of storage. This continues to be the case after increasingly long periods of storage.

In the dried milk from the drum process the decrease is not as great, relatively, as in the spray process powders. There is apparently a greater variety of organisms that have resisted drying originally in the spray process. Those which remain in the drum process product after the exposure to the high temperatures of the drum are probably principally spore-forming types which are able to withstand the conditions during storage. Unreported studies of the organisms present in dried milks after

several years of storage reveal largely Gram-positive, spore-forming rods, although some coccus-forms persist.

Table 5 presents the percentage reduction in count in the various groups of dried milks during the six-year period. The data point out clearly the high death rate during the first year.

*Types of bacteria present in dried milk*

A study was also made upon the percentage distribution of various types of organisms in the different groups of dried milk at the time the original analysis was made. The figures represent

TABLE 7  
*Effect of storage temperatures upon the bacterial content of dried milk*

TYPE OF DRIED MILK	TEMPERATURE OF STORAGE	NUMBER OF BACTERIAL COLONIES PER GRAM		REDUCTION
		Original	After 1 year	
	°C.			per cent
Spray (atomized).....	5	33,500	4,100	87.7
	10		1,500	95.2
	20		1,200	96.4
	37		900	97.3
Spray (centrifugal).....	5	27,600	3,700	86.6
	10		2,000	92.7
	20		1,500	94.5
	37		1,000	96.4
Drum.....	5	2,200	50	96.8
	10		100	95.4
	20		100	95.4
	37		50	96.8

the averages for all samples. The differentiation of types is based upon the action of the organisms upon litmus milk incubated at 20°C. for five days plus two days at 37°C. All the colonies upon the plates showing counts of 20 to 200 were picked for each sample unless the spreaders made it impossible to do so. In this case a representative plate, not showing spreaders, was selected.

The data given in table 6 indicate a state of affairs which might be expected from a knowledge of the different processes of manu-

facture. The drum process dried milk shows a predominance of peptonizing types, the spray process, of acid producing types.

It may be added that the reconstituted samples were allowed to stand in a refrigerator for two weeks after they were prepared. The majority of these showed an acid coagulation followed by peptonization, and sometimes extensive gas formation. A few of the samples remained neutral or became alkaline in reaction. In every case peptonization took place.

*Effect of storage temperatures upon the bacterial content of dried milk*

Three samples of dried milk, representing respectively the atomized spray, centrifugal spray, and drum process were divided into four lots, and stored in sealed porcelain jars for one year at 4°C., 10°C., 20°C., and 37°C. respectively.

The results are given in table 7. It will be noted that in general the samples stored at 37°C. showed the greatest reduction in count while those kept at 5°C. showed the least reduction. This is especially true of the spray process powders. There is not as much difference in the drum process samples probably because the resistant types of bacteria are the ones to survive originally the drying process.

SUMMARY

1. The bacterial counts of 31 samples of dried milk prepared by the spray process, and 13 samples prepared by the drum process are reported, before and after storage periods up to six years.
2. The original counts on the spray process samples ranged from 4400 to 5,500,000 per gram, with the majority showing counts above 50,000.
3. The original counts on the drum process samples ranged from 40 to 7,900 per gram, with the majority showing counts below 500.
4. A remarkable decrease in counts occurred in both types of dried milk after a storage period of one year.
5. The spray process powders showed a more marked reduction in counts after a two-year period of storage than did the drum

process powders. The maximum reduction in counts (99.9 per cent) for the spray process powders took place after five years. In the case of the drum process samples the maximum (97.9 per cent) was reached after three years.

6. There was a significant difference in the types of bacteria present in the dried milk prepared by the two processes.

7. High temperatures seemed to be more effective in bringing about reductions in counts during storage.

#### REFERENCES

- (1) Anonymous: Report on the Composition and Dietetic Value of "Ambrosia." Jour. State Med., London, xxx, 540-6 (1922).
- (2) Courts, F. J. H.: Reports to the Local Government Board: 1. Upon an Inquiry as to the Dried Milks with Special Reference to Their Use in Infant Feeding. Appendix: B. Reports on the Bacteriological Examination of Samples of Dried Milks by the Lister Institute of Preventive Medicine. Rpts. to Local Govt. Bd. on P. H. & Med. Subj. (London), Food Rpts., 24, 1920.
- (3) DELFINE, S.: Report to the Local Government Board upon the Effects of Certain Condensing and Drying Processes Used in the Preservation of Milk upon Its Bacterial Contents. Rpt. to Local Govt. Bd. on P. H. & Med. Subj. (London), Food Rpts., 21, 1914.
- (4) Downs, P. A.: Bacterial Flora of Milk Powder. Thesis, Cornell University Library, 1920. Quoted by Supplee and Ashbaugh (11).
- (5) GROSSO, G.: Bakteriologische Untersuchungen von Trockenmilch. Ztschr. f. Fleisch- und Milchhygiene, xvii, 312-15 (1907).
- (6) HOFFMAN, W.: Werden bei der Herstellung der Trockenmilch nach dem Just-Hatmakerschen Verfahren Rindertuberkelbazillen abgetötet? Arch. f. Hyg., lix, 216-223 (1906).
- (7) HUEFFE, F.: Über Trockenmilch. Centralbl. f. Bakt., etc. I Abt. Orig., lxi, 34-44 (1912).
- (8) JEPHOOTT, H., HUNWICK, R. F., AND RATCLIFFE, N.: The Attainment of Bacterial Purity in the Manufacture of Dried Milk. Proc. World's Dairy Congress (Washington), ii, 1265-1271 (1923).
- (9) KOSOWICZ, A.: Untersuchungen über den Bakteriengehalt der Trockenmilch. Ztschr. Landw. Versuchswesen. Österreich., xi, 719-24 (1908). Abstract in Chem. Zentbl., ii, 1531 (1908).
- (10) PRACHFELD, F.: Bakteriologische Untersuchung einiger Trockenmilchpräparate. Molk. Ztg. (Berlin), xviii, 2-3 (1908).
- (11) SUPPLEE, G. C., AND ASHBAUGH, V. J.: Bacterial content of milk powder. Jour. Dairy Sci., v, 216-228 (1922).
- (12) TILLMANS, J., AND STROHECKER, R.: Über Krause-Milchpulver. Ztschr. für Untersuch. d. Nahr. u. Genussm., xlvii, 377-420 (1924).

## PROCEEDINGS OF THE ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The annual meeting of the American Dairy Science Association was held at the University of Wisconsin, Madison, Wisconsin, on June 26, 27 and 28. The program committee consisted of H. A. Ruehe of Illinois, C. H. Eckles of Minnesota, and C. C. Hayden of Ohio, who arranged to carry out a program in a similar manner to that presented last year at the summer meeting in Michigan.

The meeting began at 9:15 on June 26 with Professor H. C. Jackson of Wisconsin presiding instead of President G. C. White of Connecticut who was unable to attend. It was fitting that Professor Jackson should have presided at this meeting and that Dean H. L. Russell should have presented the opening remarks since the first dairy school in America was held at the University of Wisconsin for the purpose of giving instruction in the Babcock test. The building in which the meeting was held was an annex to the first dairy building constructed in this country.

During the morning's session communications were presented from President G. C. White, Vice-President A. C. Baer, Secretary-Treasurer J. M. Sherman, and the chairman of the program committee H. A. Ruehe, advising the Association of their inability to attend the meeting and expressing their hope that the program would prove interesting and profitable. The meeting was unusual on account of the absence of the executive committee.

At noon the members of the Association were given a luncheon at the experimental farms of the Quaker Oats Company. Before returning to the University, the experiments in feeding oat hulls to dairy cattle and other farm live stock were explained to the visitors.

The afternoon's session was devoted principally to nutrition studies. E. L. Anthony of West Virginia presided at the meeting. At the conclusion of this meeting the members of the Association



were taken to the experimental barns of the University of Wisconsin in which a new experiment on nutrition and contagious abortion is being conducted. About 50 Holstein heifers were raised on a poor and a good dairy ration, according to E. B. Hart of the Division of Agricultural Chemistry, and these heifers, now in their first period of lactation, are being fed on these widely different rations. Within another year after the heifer production records are available, the cattle will be infected with contagious abortion for the purpose of determining the effect of nutrition upon the degree of infection from this disease.

On the evening of the same day, a banquet was planned at the Park Hotel for 6:30. A brief entertainment was given during the course of the dinner followed by several very short addresses. The real feature of the banquet was the presence of Dr. S. M. Babcock, emeritus professor of the University and originator of the test for fat in milk and cream which bears his name.

The banquet was adjourned early in the evening to permit the holding of committee meetings. The executive committee with associate editors and officers of the various sections met for the purpose of transacting necessary business. The meeting was attended by Ragsdale, Price, Anthony, Williams, Hastings, Lucas, Baltzer, and Dahlberg. Dahlberg acted as chairman and Price as secretary. The committee considered certain matters concerning publication of the JOURNAL OF DAIRY SCIENCE and the selection of committees on nominations and resolutions.

The general session held on the morning of June 27 was the last general meeting prior to the division of the membership into its various sections. In the absence of A. C. Baer of Oklahoma, A. C. Dahlberg of Geneva presided. The following committees were named.

#### NOMINATING

E. S. Guthrie, chairman  
P. S. Lucas  
A. C. Ragsdale

#### RESOLUTIONS

E. L. Anthony, chairman  
A. C. Ragsdale  
A. C. Baltzer  
W. V. Price

Resolutions were presented at the close of this session and the nominating committee gave the following report.

President.....	{ G. C. White of Connecticut J. R. Dice of North Dakota
Vice-President.....	{ H. W. Gregory of Indiana H. A. Ruehe of Illinois

The offices of secretary-treasurer and editor being held for two years, were not vacant at this time.

The dairy manufacturers section held its meeting on the afternoon of June 27 with W. V. Price of New York presiding and its meeting on the morning of June 28 with H. C. Jackson of Wisconsin presiding. One of the interesting features of this program was the report of the research committee of the International Association of Ice Cream Manufacturers given by H. F. Judkins of Springfield, Mass. This committee would welcome the coöperation of any investigator in agricultural college work who is interested in a problem of value to the ice cream industry. This committee would be glad to furnish research problems with brief outlines concerning the methods of attack or would be glad to go over existing problems for the purpose of assisting the investigator in arriving at conclusions of value to the industry. The committee would also be willing to read and comment upon manuscripts giving the results of investigational work with the intention of offering suggestions from the standpoint of the industry. Such active coöperation of an industry is especially gratifying to members of the Association and the dairy manufacturers section went on record as approving most heartily this type of coöperation. The following officers were elected for the ensuing year.

Chairman.....	W. H. Martin of Kansas
Secretary.....	J. C. Hening of New York

The sections of Production, Extension, and Official Testing held joint sessions with G. A. Williams presiding on Wednesday and A. C. Ragsdale on Thursday. The last two sections men-

tioned voted to continue their present officers while the Production section elected the following officers:

Chairman.....Fordyce Ely of Kentucky  
Secretary.....R. B. Becker of Oklahoma

The following program was presented:

#### GENERAL SESSION

*H. C. Jackson, presiding*

Opening remarks—Responsibilities of dairy staffs

Dean H. L. Russell, Univ. of Wisc.

President's address.....G. C. White, Conn. Agr. College

Announcements

Coming educational problems in dairying

Director K. L. Hatch, Univ. of Wisc.

The curricula on dairying.....H. A. Bendixen, Wash. State College

Teaching dairy cattle judging.....Fordyce Ely, Univ. of Ky.

Correspondence courses in dairy husbandry.....J. R. Dice, N. D. Agr. College

Methods of teaching dairy cattle management....H. P. Davis, Univ. of Nebr.

*E. L. Anthony, presiding*

An economic research program for the dairy industry

F. A. Buechel, U. S. D. A., Bureau of Agr. Economics

The relative anti-neuritic and anti-pellagra potency of cow's milk

W. E. Krauss and C. H. Hunt, Ohio Exp. Station

Effects of feeding vitamin A deficient rations to dairy heifers

S. I. Bechel, Pa. Exp. Station

Vitamin E experiments with different feeds.....H. P. Davis, Univ. of Nebr.

A chemical study of fermentation as applied to dairy feeds

A. E. Perkins, Ohio Exp. Station

Nutrients required for normal growth in dairy heifers

T. W. Gullickson, Univ. of Minn.

*A. C. Dahlberg, presiding*

Calcium assimilation as indicated by bone analysis in long time experiments

E. B. Meigs and A. Hartman, U. S. D. A.

A comparison of cane and kafir silage in dairy rations

R. B. Becker, Okla. A. and M. College

From what age can we raise calves on a dry grain ration

C. B. Bender, N. J. Agr. College

Management as a factor in abortion elimination from dairy herds

H. O. Henderson and E. L. Anthony, W. Va. College of Agr.

The effect of heavy concentrate feeding on the health of dairy cattle

C. F. Huffman, Mich. Agr. College

Value of rice by-products for dairy cows.....H. E. Dvorachek, Univ. of Ark.

Grinding of roughage for dairy cows.....T. M. Olson, S. D. Agr. College

Use of soybean hay and soybean meal in the dairy ration and their effect upon the quality of dairy products produced

W. B. Nevens and P. H. Tracy, Univ. of Ill.

Fetal development of the mammary gland in cattle

C. W. Turner, Univ. of Mo.

The mammary gland and milk secretion.....W. W. Swett, U. S. D. A.

The character of the material on the surface of the fat particle of milk

W. E. Petersen, Univ. of Minn.

#### DAIRY MANUFACTURERS SECTION

*W. V. Price, presiding*

Activities of the Research Committee of the International Association of Ice Cream Manufacturers

H. F. Judkins, General Ice Cream Corp., Springfield, Mass.

The relation of preheating and homogenization to the heat stability of cream

C. E. Holm, B. H. Webb, and E. F. Deysher, U. S. D. A.

Cream feathering.....P. H. Tracey, Univ. of Ill.

Does pasteurization make the mineral content of milk less assimilable

M. J. Prucha, Univ. of Ill.

The application of steam for heating and sterilizing dairy equipment

A. W. Farrell, Univ. of Calif.

The effect of hydrogen ion concentration on time of churning cream

E. S. Guthrie and P. F. Sharp, Cornell Univ.

Discussion on the judging of ice cream.....H. F. Judkins

*H. C. Jackson, presiding*

The effect of different homogenizing pressures on ice cream mix and resulting ice cream, when the percentage of butterfat is varied

W. H. E. Reid, Univ. of Mo.

The use of dry skim milk in ice cream

W. V. Price and R. Whitaker, Cornell Univ.

The effect of certain salts on the properties of ice cream mixes

J. C. Hening, N. Y. Agr. Exp. Station

The grading of commercial gelatine and its use in the manufacture of ice cream

A. C. Dahlberg, N. Y. Agr. Exp. Station

The freezing out of flavors in ice cream.....P. S. Lucas, Mich. Agr. College

A suggested rapid method for determining fat in ice cream

O. F. Garrett, Univ. of Ill.

Business session of dairy manufacturer's section

#### EXTENSION, PRODUCTION, AND OFFICIAL TESTING SECTIONS

*G. A. Williams, presiding*

State and county scrub bull eradication campaign

L. A. Higgins, Miss. A. and M. College

Discussion led by Ed. Prewitt, College of Agr., Lexington, Ky.

Missouri better bull program.....M. J. Regan, Univ. of Mo.

Organisation and operation of bull associations

S. J. Brownell, State College, Pa.

Developing a state program for proving and retaining dairy sires

E. T. Wallace, Purdue Univ.

Discussion led by George Girrbach, Mich. State College

Report of short cut methods in testing.....S. R. Searles, Univ. of Minn.

Tester training and conferences.....A. C. Baltzer, Mich. State College

Discussion led by Burt Oderkirk, Iowa State College

*A. C. Ragsdale, presiding*

Improved supervision to increase number of Dairy Herd Improvement Associations.....E. A. Hansen, Univ. of Minn.

Getting the most out of Dairy Herd Improvement Association results

A. J. Cramer, Univ. of Wisc.

Methods employed and results obtained from record tabulations

J. B. Parker, Bureau of Dairying, U. S. D. A.

The breed association herd test as it applies to Dairy Herd Improvement Associations.....O. E. Reed, Mich. State College

Developing a state wide calf club program.....M. L. Flack, Univ. of Nebr.

Dairy alfalfa campaigns.....R. H. Addy, Mich. State College

Business session of Extension, Production and Official Testing Sections

## INDEX TO VOLUME XI

<p><b>A</b>BORTION, Bovine infectious. Increased productivity of an abortion-free dairy herd..... 359</p> <p>Abstracts of some papers presented at the annual meeting..... 102</p> <p>ADDINGTON, L. H., REED, O. E., and HUFFMAN, C. F. Cotton- seed meal as a feed for dairy calves..... 488</p> <p>ALBUS, W. R., and AYERS, S. HENRY. Gassy fermentations in reheated or processed products containing pimentos..... 175</p> <p>American Dairy Science Associa- tion, Proceedings of the annual meeting of the..... 527</p> <p>American Dairy Science Associa- tion, Special meeting of the.... 111</p> <p>Announcement..... 116</p> <p>ANTHONY, ERNEST L. Butter versus oleomargarine in ricket control in pigs..... 66</p> <p>ARCHIBALD, J. G. Calf meal studies. I. Laboratory experi- ments in the improvement of physical condition. II. Feed- ing experiments with cooked and uncooked meal..... 119</p> <p>AYERS, S. HENRY, and ALBUS, W. R. Gassy fermentations in re- heated or processed cheese products containing pimentos... 175</p> <p><b>B</b>ACTERIA in butter, Distribu- tion and growth of..... 446</p> <p>Bacteria, Destruction of botuli- num toxin by milk..... 352</p> <p>Bacterial action in the coagulation of evaporated milk..... 46</p> <p>Bacterial content of dried milk, Some observations on the..... 516</p>	<p>Bacterial content of gelatin, The effect of hydrogen ion concentra- tion on the..... 313</p> <p>Bacterial content of orange sher- bet, The..... 404</p> <p>Bacterial count in clean milk com- petitions, A method of awarding marks for uniformity of low.... 155</p> <p>Bacterial counts of raw and pas- teurized milk, The influence of the pH of agar media upon the. 401</p> <p>BATEMAN, GEORGE MONROE, and SHARP, PAUL FRANCIS. Some observations on the consistency of cream and ice cream mixtures. 380</p> <p>BECHDEL, S. I., and KRUGER, J. H. Studies in the normal depo- sitions of minerals in the bones of dairy calves..... 24</p> <p>BELL, R. W., PETER, P. N., and JOHNSON, WM. T., JR. A method of obtaining crude milk sugar and other solids from sweet whey..... 163</p> <p>Book reviews..... 331</p> <p>Botulinum toxin by milk bacteria, Destruction of..... 352</p> <p>Bovine infectious abortion. In- creased productivity of an abor- tion-free dairy herd..... 359</p> <p>BOYSEN, H. H., and RAHN, OTTO. Distribution and growth of bac- teria in butter..... 446</p> <p>BURKE, A. D., WOODSON, FRANK, and HELLER, V. G. The pos- sible toxicity of buttermilk soured in zinc containers..... 79</p> <p>Butter, Distribution and growth of bacteria in..... 446</p> <p>Butter from the same churning in relation to working, Variability in composition of..... 2</p>
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- Butter salts, Studies on..... 333
- Butter versus oleomargarine in ricket control in pigs..... 66
- Butterfat determinations, The effect of lecithin in dairy products upon..... 429
- Butterfat production of offspring in dairy cattle, The effect of the ages of sire and dam on the average..... 1
- Buttermilk by ether extraction methods, Lipins and sterols as sources of error in the estimation of fat in..... 270
- Buttermilk soured in zinc containers, The possible toxicity of.. 79
- C**ALF meal studies. I. Laboratory experiments in the improvement of physical condition. II. Feeding experiments with cooked and uncooked meal..... 119
- Calves, Studies in the normal depositions of minerals in the bones of dairy..... 24
- CHALMERS, COWPER H. A method of awarding marks for uniformity of low bacterial count in clean milk competitions..... 155
- CHAPMAN, O. W. The effect of lecithin in dairy products upon butter-fat determinations..... 429
- Cheese, A comparison of the volatile-solvent method with the vacuum-oven method for determining moisture content of.. 325
- Cheese: Injury of cheese by freezing, The freezing point of Cheddar..... 9
- Cheese, The effect of flash pasteurization of milk upon the flavor and texture of Cheddar.. 292
- Cheese-making, A comparison of three methods of pasteurizing milk for cheddar..... 69
- Cheese products containing pimentos, Gassy fermentations in reheated or processed..... 175
- Coagulation of evaporated milk, Bacterial action in the..... 46
- Cocci which survive pasteurization, A study of the yellow.... 18
- COCKEFAIR, E. A. The activity of the mammary gland as determined by analysis of mammary blood before and after milking..... 230
- Colony formation upon petri plates, The effect of distilled water upon the tendency to.... 436
- Composition of milk and butter, The relation of soybean hay and ground soybeans to flavor and.. 479
- Consistency of cream and ice cream mixtures, Some observations on the..... 380
- CONVERSE, H. T. The value of silage in the experimental ration. 179
- CORDES, W. A. Bacterial action in the coagulation of evaporated milk..... 46
- CORDES, W. A., HUNZIKER, O. F., and NISSEN, B. H. Studies on butter salts..... 333
- Cottonseed meal as a feed for dairy calves..... 488
- Courses in dairy husbandry, The first..... 35
- Cream and ice cream mixtures, Some observations on the consistency of..... 380
- Cream, as affected by different homogenization pressures and different temperatures of forewarming, The heat stability and feathering of sweet..... 243
- Cream at various temperatures, Physical constants of the milk as influencing the centrifugal separation of..... 259
- Cream layers forming on Holstein and Jersey milk, The volume of the..... 409
- Cream, Physical factors influencing the formation and fat content of gravity..... 189

- DAHLBERG, A. C., and MAR-  
GUARDT, J. C.** The volume of  
the cream layers forming on  
Holstein and Jersey milk..... 409
- DAHLBERG, A. C.** Book reviews.. 331
- Dairy Notes**..... 117
- Dam** on the average butterfat  
production of offspring in dairy  
cattle, The effect of the ages of  
sire and..... 1
- DAVIS, H. P.,** The first courses in  
dairy husbandry..... 35
- Destruction** of botulinum toxin by  
milk bacteria..... 352
- Distilled water** upon the tendency  
to colony formation upon petri  
plates, The effect of..... 436
- Distribution** and growth of bac-  
teria in butter..... 446
- DOW, ODESSA D., FLANIGAN, G.  
E., and SUPPLEE, G. C.** The  
concentrated water soluble frac-  
tion of milk as a source of  
vitamin B..... 420
- Dried milk,** Some observations on  
the bacterial content of..... 516
- ELTING, ERWIN C., and GIFF-  
FORD, WARREN.** The effect of  
the ages of sire and dam on the  
average butterfat production of  
offspring in dairy cattle..... 1
- Evaporated milk** as affected by  
mixing different grades of raw  
milk, Heat coagulation of..... 471
- Evaporated milk,** Bacterial action  
in the coagulation of..... 46
- FABER, JACK.** The influence of  
the pH of agar media upon the  
bacterial counts of raw and  
pasteurized milk..... 401
- FAY, A. C., and MARTIN, W. H.,**  
The ice cream scoring contest as  
a means of improving the quality  
of ice cream..... 136
- FAY, A. C.** The bacterial content  
of orange sherbet..... 404
- FAY, A. C.** The effect of hydro-  
gen ion concentration on the  
bacterial content of gelatin..... 313
- Feathering** of sweet cream, as  
affected by different homogeni-  
zation pressures and different  
temperatures of forewarming,  
The heat stability and..... 243
- Feed** for dairy calves, Cottonseed  
meal as a..... 488
- Feeding experiments** with cooked  
and uncooked meal, Calf meal  
studies I. Laboratory experi-  
ments in the improvement of  
physical condition II..... 119
- Fermentations** in reheated or  
processed cheese products con-  
taining pimentos, Gassy..... 175
- FLANIGAN, G. E., SUPPLEE, G. C.,  
and DOW, ODESSA D.** The con-  
centrated water soluble fraction  
of milk as a source of vitamin  
B..... 420
- Flavor** and composition of milk  
and butter, The relation of soy-  
bean hay and ground soybeans  
to..... 479
- FRANDSEN, J. H.** Announcement. 116
- FRAZIER, WILLIAM C.** A defect in  
milk due to light..... 375
- Freezing point** of Cheddar cheese:  
Injury of cheese by freezing,  
The..... 9
- GASSY** fermentations in reheated  
or processed cheese products  
containing pimentos..... 175
- Gelatin,** The effect of hydrogen ion  
concentration on the bacterial  
content of..... 313
- GIFFORD, WARREN, and ELTING,  
ERWIN C.** The effect of the ages  
of sire and dam on the average  
butterfat production of offspring  
in dairy cattle..... 1
- Ghee,** The keeping qualities of... 143
- Growth** in weight of Guernsey  
cows after the age of two years. 265



- Guernsey cows after the age of two years, Growth in weight of..... 265
- HAMMER, B. W., and SARKARIA, R. S.** Influence of temperature on the changes produced in milk by certain bacteria..... 89
- HAMMER, B. W., and TROUT, G. MALCOLM.** A study of the yellow cocci which survive pasteurization..... 18
- HAMMER, B. W., and PATIL, VISHRAM, H.** The keeping qualities of ghee..... 143
- HARRIS, L. S., and WEINZIEL, JOHN.** A comparison of certain methods for determining the sanitary quality of ice cream... 284
- Heat coagulation of evaporated milk as affected by mixing different grades of raw milk..... 471
- Heat stability and feathering of sweet cream, as affected by different homogenization pressures and different temperatures of forewarming, The..... 243
- HELLER, V. G., BURKE, A. D., and WOODSON, FRANK.** The possible toxicity of buttermilk soured in zinc containers..... 79
- HENING, J. C.** Effect of homogenizing ice cream mixes before and after the addition of gelatin or sugar and before and after condensing..... 299
- HOLM, GEORGE E.** Book reviews. 331
- HOLM, GEORGE E., and WEBB, BYRON H.** The heat stability and feathering of sweet cream, as affected by different homogenization pressures and different temperatures of forewarming..... 243
- Homogenization pressures and different temperatures of forewarming, The heat stability and feathering of sweet cream, as affected by different..... 243
- Homogenizing ice cream mixes before and after the addition of gelatin or sugar and before and after condensing..... 299
- HUFFMAN, C. F., REED, O. E., and ADDINGTON, L. H.** Cottonseed meal as a feed for dairy calves.. 488
- HUNZIKER, O. F., CORDES, W. A., and NISSEN, B. H.** Studies on butter salts..... 333
- Hydrogen ion concentration on the bacterial content of gelatin, The effect of..... 313
- (Hydrogen ion concentration) pH of agar media upon the bacterial counts of raw and pasteurized milk, The influence of..... 401
- ICE cream, A comparison of certain methods for determining the sanitary quality of..... 284**
- Ice cream mixes before and after the addition of gelatin or sugar and before and after condensing, Effect of homogenizing..... 299
- Ice cream mixtures, Some observations on the consistency of cream and..... 380
- Ice cream scoring contest as a means of improving the quality of ice cream, The..... 136
- JOHNSON, WM. T., JR., BELL, R. W., and PETER, P. N.** A method of obtaining crude milk sugar and other solids from sweet whey..... 163
- JOHNSON, ROBERT E., RETTGER, LEO F., McALPINE, JAMES G., and WHITE, GEORGE C.** Bovine infectious abortion. Increased productivity of an abortion-free dairy herd..... 359
- KRUGER, J. H., and BECHEL, S. I.** Studies in the normal depositions of minerals in the bones of dairy calves..... 24

- LAWLER, B. M., and MUDGE, C.**  
 S. The effect of distilled water upon the tendency to colony formation upon petri plates..... 436
- Lecithin** in dairy products upon butterfat determinations, The effect of..... 429
- Light**, A defect in milk due to.... 375
- Lipins and sterols** as sources of error in the estimation of fat in buttermilk by ether extraction methods..... 270
- MACY, H.** Some observations on the bacterial content of dried milk..... 516
- Mammary gland** as determined by analysis of mammary blood before and after milking, The activity of the..... 230
- MANHART, V. C.** Variability in composition of butter from the same churning in relation to working..... 52
- MARQUARDT, J. C., and DAHLBERG, A. C.**, The volume of the cream layers forming on Hostein and Jersey milk..... 409
- MARTIN, W. H., and FAY, A. C.** The ice cream scoring contest as a means of improving the quality of ice cream..... 136
- MCALPINE, JAMES G., WHITE, GEORGE C., JOHNSON, ROBERT E., and RETTGER, LEO F.** Bovine infectious abortion. Increased productivity of an abortion-free dairy herd..... 359
- Milk** as influencing the centrifugal separation of cream at various temperatures, Physical constants of the..... 259
- Milk** by certain bacteria, Influence of temperature on the changes produced in..... 89
- Milk competitions**, A method of awarding marks for uniformity of low bacterial count in clean.... 155
- Milk** due to light, A defect in.... 375
- Milk sugar and other solids** from sweet whey, A method of obtaining crude..... 163
- Minerals** in the bones of dairy calves, Studies in the normal depositions of..... 24
- Moisture content** of cheese, A comparison of the volatile-solvent method with the vacuum-oven method for determining... 325
- Moisture in dry skim milk** by the Bidwell-Sterling Toluene Distillation method, The determination of..... 240
- MUDGE, C. S., and LAWLER, B. M.** The effect of distilled water upon the tendency to colony formation upon petri plates..... 436
- NELSON, J. A.** A study of the "common white" yeasts found in dairy products..... 397
- NEVENS, W. B., and TRACY, P. H.** The relation of soybean hay and ground soybeans to flavor and composition of milk and butter... 479
- NISSEN, B. H., HUNZIKER, O. F., and CORDES, W. A.** Studies on butter salts..... 333
- PASTEURIZATION**, A study of the yellow cocci which survive. 18
- Pasteurization** of milk upon the flavor and texture of Cheddar cheese, The effect of flash..... 292
- Pasteurizing milk** for Cheddar cheese-making, A comparison of three methods of..... 69
- PATIL, VISHRAM H., and HAMMER, B. W.** The keeping qualities of ghee..... 143
- PETER, P. N., BELL, R. W., and JOHNSON, WM. T., JR.** A method of obtaining crude milk sugar and other solids from sweet whey..... 163

- PETERSON, W. E., and THURSTON, L. M. Lipins and sterols as sources of error in the estimation of fat in buttermilk by ether extraction methods..... 270
- PHILLIPS, C. A. The effect of flash pasteurisation of milk upon the flavor and texture of Cheddar cheese..... 292
- Physical constants of the milk as influencing the centrifugal separation of cream at various temperatures..... 259
- Physical factors influencing the formation and fat content of gravity cream..... 189
- PRICE, WALTER, V., and PRICKETT, PAUL S. A comparison of three methods of pasteurizing milk for Cheddar cheese-making.... 69
- PRICKETT, PAUL S., and PRICE, WALTER V. A comparison of three methods of pasteurizing milk for Cheddar cheese-making. 69
- QUALITY of ice cream, The ice cream scoring contest as a means of improving the..... 136
- RAHN, OTTO, and BOYSEN, H. H. Distribution and growth of bacteria in butter..... 446
- REED, O. E., HUFFMAN, C. F., and ADDINGTON, L. H. Cottonseed meal as a feed for dairy calves.. 488
- RETTGER, LEO F., McALPINE, JAMES G., WHITE, GEORGE C., and JOHNSON, ROBERT E. Bovine infectious abortion. Increased productivity of an abortion-free dairy herd..... 359
- Ricket control in pigs, Butter versus oleomargarine in..... 66
- SALTS, Studies on butter..... 333
- SANDERS, GEORGE P. A comparison of the volatine-solvent method with the vacuum-oven method for determining moisture content of cheese..... 325
- Sanitary quality of ice cream, A comparison of certain methods for determining the..... 284
- SARKARIA, R. S., and HAMMER, B. W. Influence of temperature on the changes produced in milk by certain bacteria..... 89
- Separation of cream at various temperatures, Physical constants of the milk as influencing the centrifugal..... 259
- SHARP, PAUL FRANCIS, and BATEMAN, GEORGE MONROE. Some observations on the consistency of cream and ice cream mixtures. 380
- SHARP, PAUL FRANCIS, and TROY, H. C. Physical factors influencing the formation and fat content of gravity cream..... 189
- SHARP, PAUL FRANCIS. Physical constants of the milk as influencing the centrifugal separation of cream at various temperatures.. 259
- Sherbet, The bacterial content of orange..... 404
- SHERMAN, J. M., STARK, C. N., and STARK, PAULINE. Destruction of botulinum toxin by milk bacteria..... 352
- Silage in the experimental ration, The value of..... 179
- Sire and dam on the average butter fat production of offspring in dairy cattle, The effect of the ages of. .... 1
- Skim milk by the Bidwell-Sterling Toluene-Distillation method, The determination of moisture in dry..... 240
- SOMMER, H. H. The freezing point of Cheddar cheese: Injury of cheese by freezing..... 9
- Soybean hay and ground soybeans to flavor and composition of milk and butter, The relation of..... 479

- STARK, C. N., SHERMAN, J. M., and STARK, PAULINE. Destruction of botulinum toxin by milk bacteria. 352
- STARK, PAULINE, SHERMAN, J. M., and STARK, C. N. Destruction of botulinum toxin by milk bacteria. 352
- Sterols as sources of error in the estimation of fat in buttermilk by ether extraction methods, Lipins and..... 270
- SUPPLEE, G. C., DOW, ODESSA D., and FLANIGAN, G. E. The concentrated water soluble fraction of milk as a source of vitamin B.. 420
- TEMPERATURE** on the changes produced in milk by certain bacteria, Influence of..... 89
- THURSTON, L. M., and PETERSON, W. E. Lipins and sterols as sources of error in the estimation of fat in buttermilk by ether extraction methods..... 270
- Toxicity of buttermilk soured in zinc containers, The possible.... 79
- Toxin by milk bacteria, Destruction of botulinum..... 352
- TRACY, P. H., and NEVENS, W. B. The relation of soybean hay and ground soybeans to flavor and composition of milk and butter..... 479
- TROUT, G. MALCOLM, and HAMMER, B. W. A study of the yellow cocci which survive pasteurization..... 18
- TROY, H. C., and SHARP, PAUL FRANCIS. Physical factors influencing the formation and fat content of gravity cream..... 189
- TURNER, CHARLES W. Growth in weight of Guernsey cows after the age of two years..... 265
- VITAMIN B**, The concentrated water soluble fraction of milk as a source of..... 420
- WATER** soluble fraction of milk as a source of Vitamin B, The concentrated..... 420
- WEBB, BYRON, H., and HOLM, GEORGE E. The heat stability and feathering of sweet cream, as affected by different homogenization pressures and different temperatures of forewarming..... 243
- WEBB, BYRON H. Heat coagulation of evaporated milk as affected by mixing different grades of raw milk..... 471
- WEINZIERL, JOHN, and HARRIS, L. S. A comparison of certain methods for determining the sanitary quality of ice cream..... 284
- Whey, A method of obtaining crude milk sugar and other solids from sweet..... 163
- WHITE, GEORGE C., JOHNSON, ROBERT E., RETTGER, LEO F., and MCALPINE, JAMES G. Bovine infectious abortion. Increased productivity of an abortion-free dairy herd..... 359
- WOODSON, FRANK, BURKE, A. D., and HELLER, V. G. The possible toxicity of buttermilk soured in zinc containers..... 79
- WRIGHT, P. A. The determination of moisture in dry skim milk by the Bidwell-Sterling Toluene-Distillation method..... 240
- YEASTS** found in dairy products, A study of the "common white"..... 397



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